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EVALUATION OF REFILLABLE DRINKING WATER QUALITY BASED ON MPN *COLIFORM* AND *ESCHERICHIA COLI* IN SESETAN VILLAGE, SOUTH DENPASAR, BALI

EVALUASI KUALITAS AIR MINUM ISI ULANG BERDASARKAN MPN COLIFORM DAN ESCHERICHIA COLI DI KELURAHAN SESETAN, DENPASAR SELATAN, BALI

Research Report
Penelitian

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A B S T R A C T

Background: *Refilled drinking water given by depots that currently have a permit for operation can be used to meet the community's drinking water demands. However, the quality of refilled drinking water has been tainted with pathogens that can cause health problems.* ***Purpose:*** *To assess the quality of replenished drinking water in Sesetan Village using Most Probable Number (MPN) Coliform and Escherichia coli as indicators.* ***Method:*** *A qualitative approach to descriptive observational research. The Ministry of Health standard 492/Menkes/PER/IV/2010 is used to assess drinking water quality. In this investigation, ten samples were used. Sampling was conducted in Sesetan Village, South Denpasar, Bali.* ***Result:*** *MPN Coliform was found in four samples: 96 MPN/100 mL (X1), 38 MPN/100 mL (X7), 15 MPN/100 mL (X8), and 5 MPN/100 mL (X10). Meanwhile, all of the samples tested negative for Escherichia coli.* ***Conclusion:*** *Based on drinking water quality criteria, 4 (40%) refilled drinking water depots (DAMIU) did not meet quality requirements (Ministry of Health Regulation number 492/Menkes/PER/2010).*

A R T I C L E I N F O

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A B S T R A K

Latar belakang: Pemenuhan kebutuhan air minum dimasyarakat dapat memanfaatkan air minum isi ulang yang disediakan oleh depot yang telah memiliki izin untuk penyelengaraannya. Akan tetapi, kualitas air minum isi ulang masih ditemukan tercemar patogen yang mampu mengakibatkan gangguan pada kesehatan manusia. **Tujuan:** Mengevalusi kualitas air minum isi ulang berdasarkan *Most Probable Number (*MPN) *Coliform* dan *Escherichia coli* di Kelurahan Sesetan. **Metode:** Metode penelitian deskriptif observasional dengan pendekatan kualitatif. Evaluasi kualitas air minum menggunakan standar Kementerian Kesehatan nomor 492/Menkes/PER/IV/2010. Terdapat 10 sampel yang digunakan pada penelitian ini. Pengambilan sampel dilakukan di wilayah Kelurahan Sesetan, Denpasar Selatan, Bali. **Hasil:** Hasil penelitian menunjukkan 4 sampel mengandung MPN *Coliform* yakni 96 MPN/100 mL (X1), 38 MPN/100 mL (X7), 15 MPN/100 mL (X8), dan 5 MPN/100 mL (X10). Sedangkan seluruh sampel negatif *Escherichia coli*. **Kesimpulan:** 4 (40%) Depot Air Minum Isi Ulang (DAMIU) tidak memenuhi standar baku berdasarkan persyaratan kualitas air minum (Permenkes nomor 492/Menkes/PER/2010).

Kata kunci:
Coliform, *Escherichia coli*, Kualitas air minum isi ulang, Kelurahan Sesetan

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INTRODUCTION

Water is a lifetime supply and a vital requirement for human survival (Sudaryati *et al*., 2019). Water is utilized for various purposes, including everyday necessities and household chores. Water ingested should fulfill health requirements to maintain the safety of the entire population, including being pathogen-free, non-toxic, tasteless, odorless, transparent in color, and available at all times (Saimin *et al*., 2020). However, due to the diversity and complexity of human requirements, the water demand, particularly for drinking water, has increased (Ahmed *et al*., 2020). However, water availability, pure water, and water fit for human use are concerns (Amallia, 2020). As a result, a portion of the community relies on replenished drinking water to satisfy their daily drinking water needs (Pulungan *and* Away, 2019; String *et al*., 2021).

The conduct of individuals who are still unwilling to safeguard the environment, especially water sources, causes a reduction in the amount, location, and volume of pure water (Agista *and* Purwantisari, 2020), as a result deliberate measures are required to preserve and increase the amount and volume of water available for humanity's existence by improving the quality of refilled drinking water. The purification procedure for refillable drinking water is sought to have been passed by *Ultra Violet* (U.V.) irradiation, ozonation, or a combination of the two techniques (Uddin *et al*., 2021). Refilled drinking water must pass bacteriological, organoleptic quality, and consumption safety tests before being consumed by the general population (Wen *et al*., 2020). According to the Minister of Health of the Republic of Indonesia's Decree No. 907 of 2002, bacteriological examinations of bottled drinking water refilled drinking water, and filling stations must be conducted at least once every month and no more than once every three months (Ministry of Health, 2022).

Cleaning, filtration, and replenishing drinking water are usually available at refilled drinking water depots (DAMIU) (Kasmawati *et al*., 2020). It is hoped that there will be no pathogen contamination in the three processes and locations, which might negatively influence the quality of the generated replenishment drinking water (Morgan *et al*., 2021). According to Regulation No. 492/Menkes/PER/2010 of the Minister of Health of the Republic of Indonesia, refilled drinking water must go through several processing steps to ensure that it is free of pathogen contamination, odorless, colorless, and tasteless and that it does not endanger human health (Chaniggia *et al*., 2020; Slavik *et al*., 2020).

A previous study has indicated that many refilled drinking water bottles do not meet the Ministry of Health's quality requirements, which can show the emergence of digestive and skin illnesses that are hazardous to the population (Batubara *et al*., 2009; Sari *et al*., 2020). Agustina (2021) reported five samples of raw water from Semarang City's refill drinking water depots were positively contaminated with *Coliform* bacteria with *Most Probable Number (*MPN) values, including Candisari District (20/100 ml), Tembalang District (7/100 ml), Ngaliyan District (11/100 ml), West Semarang District (4/100 ml), and Gunung Pati District (4/100 ml), and two samples were not. Furthermore, Amallia (2020) discovered that 14 out of 20 samples of replenished drinking water did not satisfy the criteria, with the quantity of *Coliform* exceeding the quality level and five samples positive for the bacteria *Escherichia coli*. This situation demonstrates that Indonesian refilled drinking water quality is still poor and unfit for human consumption.

According to observations made in Sesetan Village, South Denpasar District, more than half of the refill drinking water depot owners failed to DAMIU procedures such as changing filters beyond the time limit, not wearing gloves and masks during production, and not conducting regular health checks at the production site. As a result, it is critical to undertake a study and assessment in the region on the safety of replenished water. In addition, previous research only focused on testing the quality of refilled drinking water in terms of the presence of pathogens in ready-to-drink water. In this study, we sought to analyze the characteristics of place and the handler associated with the presence of pathogens in the refilled drinking water produced. Based on these issues, the objective of this study was to assess the quality of replenished drinking water in Sesetan Village, South Denpasar District, Bali, using MPN *Coliform* and *Escherichia coli* criteria. Through this research, it is hoped that it can provide the latest information related to the quality of the DAMIU in Sesetan Village, Bali, and become a reference for relevant agencies in environmental governance, handling, and water production to ensure the quality of water in the community.

MATERIAL AND METHOD

Design of research

Descriptive observational research design is used to analyze and observe the study sample's procedure and quality by comparing it to standardized parameters (Darwin *et al*., 2021). The study was conducted by observing firsthand and obtaining water samples at the refilled drinking water depot, which were then examined and evaluated in the laboratory. Water sampling is carried out once during observation and visitation and without repetition.

Implementation of research

Studies were conducted for one month (August) 2020. Depot sampling locations include Depot Water Recharge (DAMIU) in the Village of Sesetan, South Denpasar District, Bali Province. Meanwhile, the water

quality test (sample) was carried out at the Quantum Medical Facilities Public Health Laboratory, with its address at Sesetan Street Number 20, Sesetan Village, South Denpasar District, Bali Province.

Determination of the population and sample

The determination of the sample in this study using purposive sampling (Darwin *et al*., 2021) took into account the owner of the refilled drinking water depot's readiness to evaluate and test samples of drinking water generated. The study population comprises 15 DAMIU scattered throughout the Sesetan village area. Meanwhile, ten refilled drinking water depots are willing to collect water samples for laboratory testing, referred to as research samples.

The tools and materials

Analytical balance, autoclave, measuring cup, incubator, becker glass, rrlenmeyer, test tube, durham tube, measuring pipette, bull filler, stirring rod, bunsen lamp, tripod, and asbestos gauze, petri disk, ose, sterile vial were the instruments utilized in this investigation. The following are the materials that were used: refilled drinking water samples, *Lactose Broth* (L.B.), *Brilliant Green Lactose Bile Broth* (BGLB), *MacConkey Agar* (MC), *Triple Sugar Iron Agar* (TSIA), *Simon Citrate Agar* (S.C.), *Sulfur Indole Motility* (SIM), Sugar Media (*Glucose, Lactose*, *Manito's*, *Maltose*, *Saccharose*) and aquadest.

The water quality sampling and testing procedures
Sampling procedure

In the Sesetan Village region of South Denpasar, Bali, refill samples of drinking water were collected. Only ten of the 15 DAMIU operational units are willing to collect water samples. During visits, a single water intake is performed, and observations are created. Water sampling is carried out by first, smoking the mouth of the faucet using a 70% alcohol cotton swab, secondly, sterilizing the mouth of the faucet with a bunsen lamp; and thirdly, carefully unscrewing the sterile bottle cap and then flowing the faucet until the water enters the bottle to taste approximately 200 mL in volume. Then, they closed the sterile bottle containing the sample tightly and gave the code; the water sample was taken to the testing laboratory for analysis.

The water quality testing procedure
The examination stage of MPN *Coliform*

The MPN *Coliform* examination in this study is based on the guidelines of the enumeration of *Escherichia coli* and the *Coliform* bacteria (Feng *et al*., n.d.) and procedures for checking refillable drinking water and requirements and supervision of drinking water quality by the Ministry of Health of the Republic of Indonesia under Number 907/Menkes/SK/VII/2002 (Ministry of Health, 2022). In the first stage, a preliminary test (presumptive test) is carried out to determine the presence or absence of *Coliform* contamination in the refilled drinking water, which is characterized by gas formation in the tube. The presumptive stages of the test include firstly, prepare five tubes each containing 10 mL of *Lactose Broth Double Strength* (tubes 1a. to 5a.). Then, prepare one tube each containing 10 mL (1b) of *Lactose Broth Single Strength* and one tube containing 10 mL of *Lactose Broth Single Strength* (1c). Furthermore, in tubes 1a to 5a, inoculated water samples of 10 mL each, tube 1b inoculated water samples of 1 mL, and tube 1c inoculated water samples of 0.1 mL. The next step is to shake the tube slowly so that the water sample evenly spreads throughout the substrate and is inoculated at 35°C for 24–48 hours. In the final stage, readings are carried out by looking at the presence or absence of gas in each tube. Suppose there is a formation of gas in the tube, in that case, it shows a favorable presumption, but this has not confirmed the presence of *Coliform* in the water because lactose broth can also be fermented by bacteria other than *Coliform*. Thus, a positive preliminary test is followed by an affirmation test.

In the second stage, an affirmative test is carried out to confirm the positive sample in the preliminary test. The affirmation test is carried out by from each positive preliminary test, transferring 1 - 2 oses into an affirmation test tube containing 10 mL of *Brilliant Green Lactose Bile Broth* (BGLB) media, from each preliminary test tube, inoculating into two BGLB tubes. One series of BGLB tubes was incubated at 37°C for 24–48 hours (to ensure the presence of *Coliform*), and another was incubated at 44°C for 24 hours (to ensure the presence of fecal *Coli*). Furthermore, after 24 or 48 hours, observations were made by looking at the number of BGLB tubes that indicated the presence of gas (positive). To determine the value of MPN, *Coliform* is carried out by calculating the number of positive tubes in the 5-1-1 tube series. The number obtained is then matched with the MPN index table. In the MPN index table are listed the tube series and sample volume. If the first five tubes are matched with a positive number, as well as the one-second tube and the one-third tube, mean *Coliform* figures will be obtained.

The *Escherichia coli* bacterial examination stage

An *E. coli* bacteria examination of refillable drinking water is carried out to ensure that these bacteria pollute no drinking water. At this stage, six tests were carried out using different media.

Testing with *Thioglycolic* media

Thioglycolate media is a bacterial growth medium used for further identification. Bacteria grow at the top (aerobic), middle (facultative aerobic), and bottom (anaerobic) of the medium, which is sterile. At this stage, prepare 10 *Thioglycolic* media and equip them with a label according to the sample number. Then, the sample was taken with a sterile 5 mL measuring pipette of 2 mL, poured into a tube containing *Thioglycolic* media according to the label, and then incubated in an incubator at a temperature of 37°C for 1×24 hours.

Testing with *MacConkey* media

MacConkey agar medium is recommended for standardized *Coliform* bacteria testing in food, plants, and water. *MacConkey agar* is a selective and different medium used to separate gram-negative stem bacteria based on whether or not they can ferment lactose. *MacConkey agar* medium is recommended for standardized *Coliform* bacteria testing in food, plants, and water. *MacConkey agar* is a selective and different medium used to separate gram-negative stem bacteria based on whether or not they can ferment lactose. At this stage, prepare *MacConkey* media in a petri disk according to the number of samples grown on *thioglycolic* media. Then a spherical ose sterilization with fire was taken, and a sample was taken that had been bred on *thioglycolate* media by dipping the ose into the *thioglycolate* and then streaking on *MacConkey* media. The next stage is to carry out incubation in an incubator for 1×24 hours at a temperature of 37°C, and at the final stage, colony growth is observed on the medium. When bacteria like *E. coli* are present, they appear as red colonies surrounded by cloudy areas.

Testing with TSIA identification media

TSIA media is used to identify gram-negative groups of bacteria characterized by their ability to ferment sugars and form *hydrogen sulfide* (H_2S). The formation of H_2S by bacterial metabolic activity is monitored through pH indicators, namely red phenol compounds and $FeSO_4$.At this stage, prepare a tube containing TSIA media according to the sample in the next *E. coli* colony, sterilize the needle ose with fire, and pierce the ose into the media, streaking on the oblique part of the TSIA media. Incubate the media in an incubator with a temperature of 37°C for 1×24 hours and conduct media observations with criteria such as the color formed on the slopes and the base of the medium, the presence of gases, and the presence of H_2S.

Testing with (*Simon Citrate Agar*) SCA identification media

Media *Simmons Microorganisms* that use citrate as a source of carbon can use citrate as a source of carbon. SCA contains sodium citrate and ammonium phosphate, each functioning as a carbon and nitrogen source. SCA is useful for distinguishing gram-negative enteric bacteria in the form of bacilli in test samples in the form of water, food, and laboratory specimens. At this stage, prepare a tube containing SCA media according to samples containing *E. coli* colonies at this stage. Next, sterilize the spherical ose with fire, then take with a red colony round ose surrounded by a turbid zone on Mac Conkey media, dispersing the media from the bottom up and incubating the media in the incubator for 1×24 hours at a temperature of 37 °C.

Testing with SIM identification media

Indole motility (SIM) sulfide is a semisolid agar medium used to determine H_2S production, *indole* formation, and the motility or movement of a bacterium. SIM media is used to distinguish members of *Enterobacteriaceae*. At this stage, prepare a tube containing as many SIM media as samples as there are *E. coli* colonies, then sterilize the needle with spritus fire, and then a red colony is taken surrounded by a turbid zone on *MacConkey* media. Puncture up to two-thirds of the SIM media parts, then incubate the media at the incubator for 1×24 hours at a temperature of 37°C, observe the motion, and add *Kovac's* reagents for *indole* testing if the positive *indole* test is marked by a red ring forming on the surface of the medium.

Testing with *glucose, lactose, saccharose, maltose,* and *manitose* identification media

Testing with sugar-sugar media aims to determine the ability of bacteria to degrade sugar and produce organic acids derived from each type of sugar, namely *glucose*, *lactose*, *saccharin*, *maltose*, and *mannitol*. At this stage, prepare a tube containing confectionery media (*glucose*, *lactose*, *sacharosa*, *maltose*, and *manitosa*), then sterilize the round ose with spritus fire, then take colonies of *E. coli* bacteria on *MacConkey* media using round ose plans on each confectionery media by stirring the ose in the media (homogenize), followed by incubation of the media in the incubator for 1×24 hours at 37°C and media observations and matching with the interpretation of expected results.

Data analysis

The data were collected from findings from the laboratory's refilled drinking water quality test, which were then evaluated using tables and narratives in a comparative descriptive way. MPN *Coliform* with MPN/100 mL units and data for *Escherichia coli* in the form of positive and negative. Furthermore, the data are compared to the drinking water quality criteria established by the Ministry of Health of the Republic of Indonesia under number 492/MENKES/PER/2010 for Drinking Water Quality Requirements in the Republic of Indonesia.

RESULT

Various findings on the MPN of *Coliform* and *Escherichia coli* values were obtained based on the results of a study linked to the assessment of the quality of refilled drinking water at ten depots in the Sesetan Village area, South Denpasar District, Bali Province. According to the results of laboratory tests (Table 1), there are four (40%) refilled drinking water

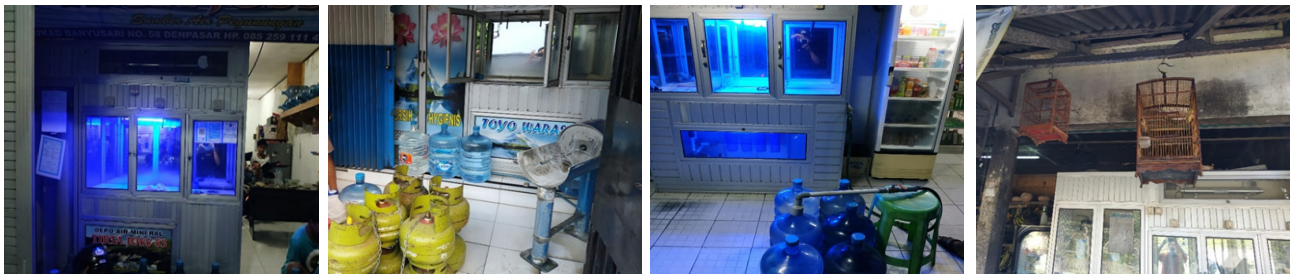
depots in Sesetan Village, South Denpasar District, Bali Province, that do not meet the standards and exceed the MPN 0/100 mL threshold set by the Minister of Health Regulation No. 492 of 2010 (MPN 0/100 mL). X1 (MPN 96/100 mL), X7 (MPN 38/100 mL), X8 (MPN 15/100 mL), and X10 (MPN 5/100 mL) were the refill water samples that surpassed the threshold. As a result, six samples passed the established standard standards, allowing operations to be evaluated regularly, and four positive samples of MPN *Coliform* were sent for review in line with standard requirements of the Minister of Health of the Republic of Indonesia Regulation number 492/ Menkes/PER/IV/2010 concerning Drinking Water Quality Requirements. Total *Coliform* is a group of bacteria used to indicate the presence of manure pollution. *Coliform* bacteria residing in the water allow for the presence of *Enteropathogenic* microbes and are harmful to human health. Furthermore, the results of microbiological examinations on water samples that have been carried out show that no samples of refillable drinking water contaminated with *Escherichia coli* were found on examination using standard water quality testing media. This indicates that the raw water used to produce refillable drinking water is not contaminated by fecal water, which is supported by negative *Escherichia coli* results.

Table 1. MPN test value *Coliform* and *Escherichia coli*

Sample	Inspection result (MPN/100mL)		PERMENKES No 492/Menkes/PER/IV/2010		Result description	Environmental inspection results
	Coliform	*E. coli*	*Coliform*	*E. coli*		
X1	96	0	0	0	*	^*^,♣
X2	0	0	0	0	***	-
X3	0	0	0	0	***	-
X4	0	0	0	0	***	-
X5	0	0	0	0	***	-
X6	0	0	0	0	***	-
X7	38	0	0	0	*	^*^,◆
X8	15	0	0	0	*	^*^,◆
X9	0	0	0	0	***	-
X10	5	0	0	0	*	^*^, ◆

Information: * =Does not meet the quality standards of refilled drinking water; *** =Meet the quality standards of refilled drinking water; ^*^ =Do not replace the filter cartridge, ◆=Joined with other activities (selling LPG gas, gallons of water, and there is an aviary); ♣ =Does not meet DAMIU physical standards

Figure 1 depicts depots that are connected with other activities. Field observations revealed that some depots had an unsuitable atmosphere and squandered sanitation. Three of the four *Coliform*-containing depots have been identified by researchers as being connected with other activity areas such as booths, sellers of LPG gas, fruit sales, and the presence of pet birds. One of the three depots did not fulfill the physical standards, as seen by the filthy floor, moldy walls, and perforated ceiling, all of which influenced the drinking water quality. The physical space requirements for the refillable drinking water depot must be met during construction. The condition of the floor, the state of the walls, the condition of the ceiling, and the size of the room are all physical criteria. The floors, walls, and roofs of the refilled drinking water depot must be constructed of waterproof material, have a level surface, be smooth but not slippery, be dust-resistant and straightforward to clean, and be kept clean and dust-free at all times.



(a) (b) (c) (d)

Figure 1. Depots are associated with other activities

Description: a. Filling operators do not use personal protective equipment; b. Depot with LPG gas sales; c. Depot with stalls; d. Depot with a bird's nest

Depots that do not stand alone and are combined with other activity sites allow pollution from the surrounding environment. The cleanliness of the depot must be maintained at all times to avoid pathogen contamination and a reduction in the quality of the replenished drinking water. The results of direct interviews with the operator or owner of the refilled drinking water depot showed that four out of ten DAMIU do not change their water filters more than once every 1 - 2 months, do not have multilevel filters that prevent contamination, and use U.V. rays that are slightly less powerful than they should be. This means the filtration and sterilization processes are not in line with the rules.

DISCUSSION

As a human need, drinking water is a source of life (Sudaryati, 2018). The community consumes refilled drinking water daily to satisfy the availability of water consumption. The safety of replenishing drinking water is monitored regularly to guarantee that high-quality water is generated. The microorganisms that cause sickness in humans must be kept out of refillable drinking water appropriate for consumption (Ohorella *et al*., 2020). Total *Coliform* and *E. coli* were the parameters examined. The investigation results in Table 1 show that pathogens were found in refilled drinking water at a depot in the Sesetan area, South Denpasar sub-district, Bali province.

The high value of the MPN of *Coliform* in the four samples tested, namely Samples X1, X7, X8, and X10, is because the water used as refilled drinking water is sourced from groundwater and or water sources distributed using less hygienic tanks, resulting in contamination during the distribution process. The cleanliness and quality of the water used as the primary raw material determine the quality of replenished drinking water (Kato *et al*., 2022; Uddin *et al*., 2021). The primary raw material for refilled drinking water comes at least from water sources that have been guaranteed and tested for quality to produce drinking water that is suitable for consumption, safe for health, free of pathogens and other contaminants, and that is checked physically, chemically, and biologically regularly (Akhter *et al*., 2020; Winandar *et al*., 2020). According to research conducted by Najah *et al*. (2020), the quality of replenishment drinking water is determined by the quality of the raw materials utilized.

Furthermore, Puspitasari *et al*. (2020) and Sari *et al*. (2020) discovered a high value of MPN *Coliform* in refilled drinking water caused by wastewater from human waste and or household waste (laundry, bathing, washing toilets) seeping into river waters, springs, and raw water in nature, despite the weak filtration system. As a result, polluted water is classified as contaminated water. According to Ministry of Health regulations, drinking water must be free of *Coliforms*. The higher the degree of contamination with bacteria *Coliform*, the greater the danger of other diseases that reside in human and animal excrement. Hygiene and sanitation that had not been implemented according to recognized requirements contaminated four refilled drinking water filling terminals in the Sesetan Village region, South Denpasar District, Bali Province. Hygiene and sanitation are critical for maintaining health and quality and replenishing drinking water production (Evans *et al*., 2020; Saimin *et al*., 2020). *Coliform* bacteria in drinking water suggests that the process and sanitation are insufficient. Furthermore, cleaning and storing for extended periods might degrade the quality and increase the *Coliform* bacteria population in water (Wen *et al*., 2020) According to the Ministry of Industry and Trade of the Republic of Indonesia decision number 651/MPP/Kep/10/2004, refilled drinking water must meet the technical requirements of drinking water depots, which must be monitored and inspected regularly and at least once every three months for inspection laboratory (Ministry of Health, 2022; Ministry of Industry and Trade, 2004)

Regarding the discovery of indications of *Coliform* contamination in refillable drinking water, it has been confirmed through direct interviews with the owner or operator of the refilled drinking water depot that the replacement of cartridge filters for the production and filtration of refillable drinking water is not performed periodically, depending on the color and condition of the cartridge. If it is still in good condition and looks new even though it has passed the replacement time it should be (once every 1 - 2 months), then the DAMIU operator does not replace the cartridge filter to minimize replacement costs. The findings at the refilled drinking water depot that were confirmed positive for *Coliform* (4 DAMIU) all replaced the cartridge filter every >4 months, so that it became one of the factors for contamination in refilled drinking water. According to the observations and interviews with the owner and staff of the depot located in the Sesetan Village area, some staff and employees do not know when to replace the cartridge filter. This has implications for contamination in the refilled drinking water produced. These results are in line with the study Morgan *et al*. (2021) and Spanoudi *et al*. (2021); refillable drinking water depots or drinking water providers that do not replace cartridge filters periodically and do not apply multilevel filters may result in production and filtering equipment getting dirty and resulting in the presence of bacterial contamination that exceeds the established limits. The cartridge filter influences the quality of the resultant drinking water, and the short cleaning time of gallons of water with food detergent (food grade) results in gallons of water that are highly dubious in terms of cleanliness. Gallons are rinsed with clean water heated to 60 – 85^{o}C to eliminate any leftover detergent, then dried and filled with raw water (Agustina, 2021; Anthonj *et al*., 2020; Kelly *et al*., 2021).

Observations at ten depots revealed that replenishing water was carried out in multiple phases, including rinsing with water, brushing, rinsing again with water, sterilization, and finally filling with drinking water suitable for public use. Many behaviors and phases were seen that were not in line with the requirements established in the four depots that revealed positive *Coliform*, such as personal hygiene personnel and or depot employees who did not wear personal protective equipment, according to the observations (Cha *et al*., 2021). Furthermore, most staff who meet with potential clients do not wash their hands first. Employees who do not use gloves or wash their hands during cleaning risk spreading pathogens inside the gallon's tip. Furthermore, the filling operator's expertise is insufficient, and the filling location is filthy, impacting the quality of the drinking water generated. According to Najah *et al*. (2020) and Agustina (2021), the cleanliness of drinking water locations, handling of containers and refills, and the knowledge of filling operators all significantly impact the quality of refilled drinking water at the depot.

The equipment used in the manufacture of replenishment drinking water must fulfill technical criteria and be serviced regularly (Chaniggia *et al*., 2020; Pulungan *and* Away, 2019). Furthermore, sterilizing equipment determines the quality of the drinking water generated. Sterilization equipment that has outlived its usefulness cannot remove pathogens from drinking water, resulting in water contamination with the bacteria *Coliform*. Agustina (2021) explains that there are several determining factors for drinking refilled drinking water contaminated with *Coliforms*, including (1) The length of time water is stored in reservoirs so that it affects the quality of the raw water sources used; (2) The presence of contamination while entering the water into the transport tank; (3) Shelters are not clean; (4) Less than optimal processing; (5) Environmental cleanliness; (6) The presence of contamination from unsterilized gallons.

The findings of ten DAMIU and samples revealed that the bacteria *Escherichia coli* were not present. This means that six of ten refilled drinking water depots are open for business, and the water produced is safe to consume. According to Agista and Purwantisari's (2020) research, water that was not contaminated with *Escherichia coli* was indicated by appropriate depot cleanliness, requirements that had been reached, raw water that was safe for consumption, and filling operators' knowledge and conduct. Furthermore, the requirements and procedures for refilling drinking water containers at ten refilled drinking water depots have gone through several filtering stages, including a pre-filter (sand filter) to remove coarse particles, a carbon filter to absorb odors, tastes, colors, and organic matter, a cartridge filter to filter out fine particles, and U.V. filters or ozonation to disinfect drinking water (Ahmed *et al*., 2020). If these processes are not followed and carried out with care, the quality of the replenished drinking water will suffer (Akhter *et al*., 2020; Rohma *and* Meishanti, 2021; Winandar *et al*., 2020).

Based on the test results and findings, it shows that there are 40% (4 DAMIU) of the ten DAMIU included in the study located in the Sesetan Village area, South Denpasar District, Denpasar City that are still experiencing *Coliform* bacterial contamination and do not meet the requirements set by the Ministry of Health of the Republic of Indonesia and periodic evaluations are needed regarding place conditions, business feasibility, guard operator and compliance with predetermined parameters. Meanwhile, 60% (6 depots) have met the requirements of microbiologically, physically, and environmentally sound standards and are checked regularly to ensure the suitability of all indicators. This study's results align with research conducted by Puspitasari *et al*. (2020). There are still refillable drinking water depots that do not meet quality standards, and the water produced contains pathogens that can harm human health. Only three of the twenty-one DAMIU evaluated met the requirements, indicating that future evaluation regarding the quality of refilled drinking water must be improved. In addition, Kato *et al*. (2022) explain that to produce safe refilled drinking water of high quality and purity, DAMIU owners must periodically evaluate and monitor the raw water used to minimize pathogenic contamination. However, in this study, five DAMIU had not allowed their water samples to be tested in the laboratory. As a result, the Denpasar city health office and public health center that serve the village area must monitor 15 refillable drinking water DAMIU once a month and then every three months and six months, as required.

CONCLUSION

The quality of refilled drinking water in Sesetan Village, South Denpasar District, Bali, is 40% (four refilled drinking water depots) positive for *Coliform* and does not meet the standards set by the Minister of Health of the Republic of Indonesia's Regulation No. 492/Menkes/PER/IV/2010 concerning Drinking Water Quality Requirements. All of the DAMIU were found to be free of *Escherichia coli*. This study will be used as information, reference, and reference material in policymaking on DAMIU in the Sesetan Village region of the South Denpasar District of Bali Province.

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