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Original Research Paper

Microbiological Analysis of *Escherichia coli* in Refilled Drinking Water in Pariaman City

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Abstract: Drinking water is the most important substance in life after water. Drinking water containing *Escherichia coli* bacteria indicates that the water is still polluted. The purpose of this study was to determine the quality of drinking water and identify the presence of bacteria from and *E. coli* in drinking water samples. The method used in this study was a membrane filter using 4 drinking water samples. From the results of testing drinking water depot samples, it showed that the S.030a sample obtained *E. Coli* results of 13 CFU / ml, the S.031a sample obtained 1 CFU / ml, the S.032a sample obtained 0 CFU / ml and the S. 036a sample obtained 0 CFU / ml. From the drinking water examined, there were 2 samples that did not meet drinking water quality standards and there were 2 samples that met drinking water quality standards. In accordance with the Minister of Health Regulation 2 of 2023 concerning Environmental Health, the drinking water quality standard is 0 CFU / 100 ml.

Keywords: Drinking Water; *Escherichia coli;* Membrane Filter.

Introduction

Water is a fundamental human necessity, vital for domestic, industrial, and agricultural sectors, as well as for elevating public health standards. Consequently, managing both its quality and quantity is imperative. While the hydrological cycle naturally replenishes surface and marine water resources, rapid population growth and anthropogenic activities have significantly induced water pollution, thereby compromising its pristine quality (Fadila et al., 2024). The establishment of water quality standards is primary aimed at mitigating and preventing the proliferation of water-borne diseases (Azkhiyati, 2023). Furthermore, drinking water is scientifically defined as treated water that rigorously complies with established health and safety criteria, rendering it safe for human consumption (Apriani et al., 2019).

Driven by rapid population growth, the public demand for drinking water escalates continuously, outstripping the availability of adequate clean water resources. The inefficient utilization of groundwater alongside the infrastructural limitations of municipal water utilities (PDAM) has propelled the community to adopt refillable drinking water (AMIU) as a practical and economically viable alternative (Raksanagara et al., 2018). Currently, AMIU products serve as a primary choice across various socioeconomic strata, particularly among lower-middle-class households, due to their significantly higher affordability compared to commercially packaged bottled water (Widyarto et al., 2019).

A refillable drinking water depot (DAMIU) is an industrial enterprise within the beverage sector that processes raw water into potable water meeting consumption standards for direct retail to consumers. The widespread proliferation of these refillable water depots is primarily driven by their competitive pricing, offering a significantly more economical alternative to commercially branded bottled water (Narsi et al., 2017).

The implementation of the latest

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regulatory framework, specifically the Regulation of the Minister of Health of the Republic of Indonesia Number 2 of 2023, mandates that drinking water must comply with microbiological criteria, strictly enforcing a threshold of zero colonies per 100 mL of sample. Nevertheless, quality fluctuations at the depot level frequently evade routine surveillance due to limitations in rapid and precise detection methodologies (Putra & Azizah, 2020). Non-compliance with these established standards necessitates technical intervention in the form of periodic quality monitoring, utilizing methods capable of providing reliable quantitative data.

Nevertheless, the microbiological quality and nutritional aspects of refillable drinking water remain heavily scrutinized due to heterogeneous processing and distribution methods that do not consistently align with sanitary standards. Numerous refillable water depots lack adequate sanitation systems, and the reuse of water carboys without proper sterilization presents a critical vector for microbial contamination (Aulia et al., 2025).

Unstandardized management conditions of refillable drinking water depots (DAMIU) are also prevalent in Pariaman City. Concurrently with the growing number of depots established to meet public demand, periodic microbiological quality surveillance remains a significant challenge (Febria, 2022). The discrepancy between field practices and the mandatory standard of 0 CFU/100 mL poses a substantial risk to local public health. Consequently, high-accuracy and high-precision testing is critically required to guarantee consumer safety.

In this study, the detection of *Escherichia coli* was conducted utilizing the Membrane Filtration (MF) method. This microbiological technique involves filtering a specific volume of water through a membrane filter with a designated pore size to entrap bacterial cells on the membrane surface (Hardianto et al., 2022). The selection of the MF method is predicated on its advantage of providing direct colony quantification in Colony Forming Units (CFU), alongside its superior sensitivity in detecting bacterial contamination in samples with low microbial density (Sari & Wahyuni, 2022). Based on this background, research on the microbiological quality of refillable drinking water in Pariaman City was undertaken to ensure compliance with public health standards.

Material and Metods

Research Time and Location

This study was conducted from January to February 2026 at the Environmental Laboratory Technical Implementation Unit (UPTD), Department of Public Housing, Residential Areas and Environmental Affairs of Pariaman City. The research activities included the preparation of equipment and materials, sample collection and handling, laboratory examination, observation and enumeration of bacterial colonies, data analysis, and preparation of the research report.

Research Design

This study employed a descriptive laboratory-based approach aimed at determining the presence and number of coliform bacteria and *Escherichia coli* in water samples using the membrane filtration method with *Chromocult Coliform Agar* (CCA) medium. The study population consisted of all water from the sources designated as the objects of examination, while the research samples consisted of water collected from predetermined sampling points. The number of samples was determined according to the number of sampling points and types of water samples examined. Samples were collected using the grab sampling technique with reference to SNI 9063:2022.

During transportation, the samples were maintained at **(**5 ± 3) °C and examined within a maximum holding time of 24 hours after collection. The research variables included the presence and number of coliform bacteria and *E. coli* based on the characteristics of colonies growing on CCA medium. Data were obtained through laboratory examination using the membrane filtration method, followed by observation of colony characteristics and enumeration. The equipment used included an analytical balance, membrane filtration apparatus, vacuum pump, autoclave, incubator, water bath, *Laminar Air Flow* (LAF) cabinet, Petri dishes, Erlenmeyer flasks, forceps, and a magnetic stirrer. The materials consisted of water samples, *Chromocult Coliform Agar (CCA)* medium, membrane filters, and distilled water.

Research Procedures

Equipment Preparation and Sterilization

The initial stage of the study involved preparing all required equipment and materials. Equipment used for the examination, including Erlenmeyer flasks, Petri dishes, and forceps, was sterilized using an autoclave at 121 °C and 1 atm pressure for 15 minutes**.** All microbiological procedures were performed aseptically to minimize the risk of contamination.

Preparation of Chromocult Coliform Agar (CCA) Medium

Chromocult Coliform Agar (CCA) medium was prepared by weighing 26.5 g of the medium and dissolving it in 1 liter of demineralized water**.** The mixture was homogeneous stirred using a magnetic stirrer and heated at 40–45 °C until homogeneous. The homogeneous medium was then aseptically poured into sterile Petri dishes and allowed to solidify completely before use.

Sample Collection and Handling

Samples were collected using the grab sampling technique with reference to SNI 9063:2022**.** The samples were placed in appropriate sample containers and labeled according to sample identification, sampling location, and collection time. During transportation to the laboratory, the samples were maintained at (5 ± 3) °C and examined within a maximum of 24 hours after collection.

Sample Preparation

Prior to examination, the samples were homogenized, and the need for dilution was determined according to the type and condition of the sample. For drinking water samples, 50 mL of the sample was mixed with 50 mL of distilled water and homogenized. The prepared samples were subsequently subjected to membrane filtration.

Sample Filtration

Filtration is performed using a membrane filtration apparatus connected to a vacuum pump. A 100 mL sample is filtered through a sterile membrane filter. For bottled drinking water samples, a sample volume of 250 mL is used, while the minimum volume used for filtration is 10 mL. Following filtration, the membrane filter is removed aseptically using sterile forceps.

Placement of Membrane Filter on CCA Medium

After filtration, the membrane filter is placed aseptically onto the surface of the CCA medium. The membrane is positioned carefully to ensure complete contact with the medium and to prevent air bubbles from becoming trapped between the membrane and the agar surface.

Incubation

The Petri dishes containing the membrane filters were incubated in an inverted position at **(**36 ± 2) °C for (21 ± 3) hours**.** After incubation, bacterial growth and colony characteristics on the membrane surface were observed.

Colony Observation and Enumeration

Colonies growing on the CCA medium were observed based on their characteristic colors. Pink-to-red colonies, indicating β-D-galactosidase activity, were counted as presumptive coliform bacteria. Dark-blue colonies, indicating both β-D-galactosidase and β-D-glucuronidase activities, were categorized as presumptive *Escherichia coli*. Colonies exhibiting these characteristics were counted and recorded as the examination results.

Data Analysis

The examination data were analyzed descriptively based on the observation and enumeration of colonies growing on *Chromocult Coliform Agar* (CCA) medium. The data included colony color characteristics and the number of presumptive coliform bacteria and *Escherichia coli* colonies. The results were subsequently presented in tables and descriptive narratives to describe the microbiological examination results of each water sample analyzed.

Result and Discussion

Results of *Escherichia coli* Examination

A microbiological quality assessment was conducted on four refillable drinking water samples collected from several drinking water depots in Pariaman City. The examination was performed using the membrane filtration method

with *Chromocult Coliform Agar* (CCA) to determine the presence and number of *Escherichia coli* (*E. coli*) in the water samples. Positive and negative control strains were included as part of the quality control procedure. The positive control showed the expected bacterial growth, whereas the negative control showed no bacterial growth. These results indicate that the growth medium and analytical procedure were suitable for examination of the collected samples.

Each water sample was analyzed using a 100 mL analytical volume. The use of this volume allowed the results to be expressed as Colony Forming Units per 100 milliliters (CFU/100 mL), enabling direct comparison with the microbiological quality requirements applied in this study. The examination showed differences in the number of *E. coli* among the analyzed samples. Sample S.030a had the highest *E. coli* count, with 13 CFU/100 mL, whereas sample S.031a contained 1 CFU/100 mL. No *E. coli* colonies were detected in samples S.032a and S.036a, with both samples showing a result of 0 CFU/100 mL.

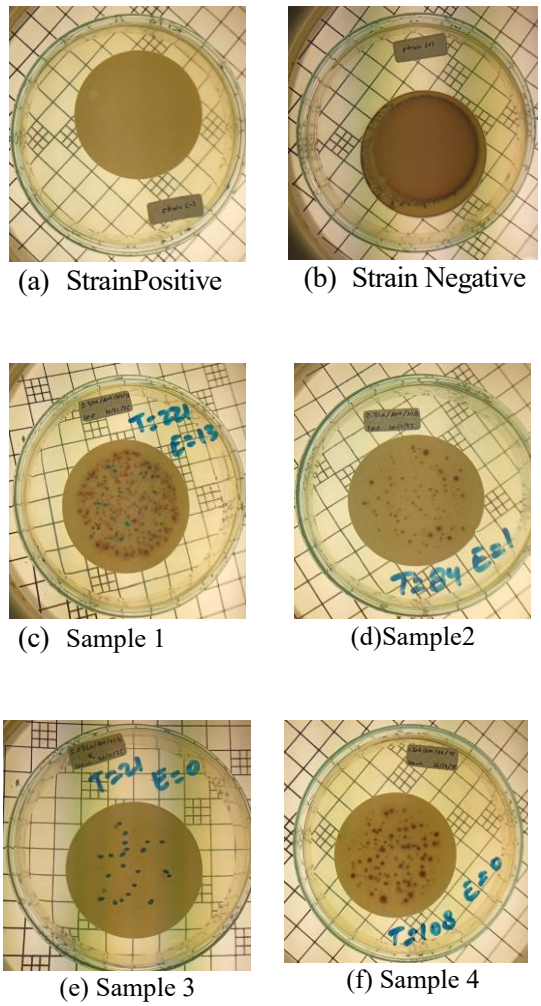
Table 1. Observation Results of *Escherichia coli* Bacteria in Water Samples

No	Sample Code	*E. coli* Count (CFU/100 mL)	Quality Standards (CFU/100 mL)
	Strain Positif	Positive	-
	Strain Negatif	Negative	-
1.	S.030a	13	0
2.	S.031a	1	0
3.	S.032a	0	0
4.	S.036a	0	0

Based on the data presented in Table 1, differences were observed in the *E. coli* results among the examined samples. Sample S.030a showed the highest colony count, at 13 CFU/100 mL, while sample S.031a showed a lower count of 1 CFU/100 mL. In contrast, no *E. coli* colonies were detected in samples S.032a and S.036a. Therefore, two of the four examined samples showed the presence of *E. coli*, while the other two samples showed no detectable *E. coli* growth.

The differences in the results indicate that the microbiological conditions of the examined samples were not uniform. Sample S.030a had the highest level of *E. coli* contamination, whereas S.031a showed a substantially lower count. The results of 0 CFU/100 mL in S.032a and S.036a indicate that no *E. coli* was detected in the examined sample volume. Based on these findings, the two samples with detectable *E. coli* require further attention because the applicable quality standard requires the absence of *E. coli* in drinking water.

Visual observations of *E. coli* colony growth in each sample are presented in Figure 1. The documentation includes the positive control strain, negative control strain, and the results obtained from the four examined water samples.



(a) StrainPositive (b) Strain Negative

(c) Sample 1 (d)Sample2

(e) Sample 3 (f) Sample 4

Figure 1. Visual documentation of assay results: (a) Positive Strain, (b) Negative Strain, (c) Sample 1, (d) Sample 2, (e) Sample 3, and (f) Sample 4.

The visual observations showed the presence of *E. coli* based on the characteristic colony appearance on CCA. The positive control showed bacterial growth consistent with the expected characteristics, whereas the negative control showed no growth. Among the study samples, colony growth was observed in S.030a and S.031a, while no *E. coli* colonies were observed in S.032a and S.036a. These visual observations support the colony counts presented in Table 1.

Overall, the findings demonstrate that *E. coli* was not detected in all examined water samples. However, the detection of *E. coli* in two samples indicates that microbiological quality problems remain present in some of the examined refillable drinking water samples. This finding is important because drinking water is consumed directly by the public, making microbiological quality a critical component of water safety.

Comparison of Findings with Previous Studies

The results of this study indicate that *E. coli* was detected in 2 out of the 4 refill drinking water samples examined. These findings demonstrate that there are still refill drinking water samples with microbiological issues. These results align with a study conducted by Putri and Priyono (2022) in Gajahmungkur District, which found contamination by indicator bacteria in the majority of tested refill drinking water samples. That study utilized the membrane filtration method and revealed that 7 out of 8 samples were contaminated with coliform bacteria. It also linked microbiological conditions to operator hygiene, environmental conditions, filter cleanliness, and the state of the depot facilities.

The findings of this study can also be compared to research by Harianja *et al.,* (2022), which examined equipment maintenance and the supervision of treatment processes at refill drinking water depots. Their study showed that *E. coli* contamination in refill drinking water is associated with equipment maintenance as well as the implementation of hygiene and sanitation practices in depot management. These findings illustrate that the microbiological quality of drinking water is influenced not only by the quality of the raw water but also by treatment methods and equipment maintenance.

These findings can also be compared with the study by Harianja *et al.,* (2022), which examined equipment maintenance and process supervision at refill drinking water depots. Their study demonstrated that *E. coli* contamination is associated with equipment maintenance as well as the implementation of hygiene and sanitation practices in depot management. These findings indicate that the microbiological quality of drinking water can be influenced not only by the quality of the raw water but also by the treatment process and equipment maintenance.

A study by Fajar *et al.,* (2023) reported E. *coli* contamination in refill drinking water in the Ciangsana area. Of the ten samples examined, two tested positive for *E. coli*. These findings follow a pattern relatively similar to the present study: not all samples tested positive, yet some showed contamination. Differences in sample size and testing methods mean the quantitative results of the two studies cannot be directly compared; however, both demonstrate that *E. coli* contamination can still be found in refill drinking water.

Research by Bandu *et al.,* (2023) on the bacteriological quality of refill drinking water in Lolak, Bolaang Mongondow, also highlights the importance of testing for *E. coli* and *coliforms* as water quality indicators. That study specifically evaluated the bacteriological quality of refill drinking water and demonstrated that microbiological testing is essential to assess whether the water produced by depots meets drinking water quality standards.

A study by Zulfa and Mulyawaty (2023) in the Gunungpati District of Semarang City offers an additional perspective, as it examined not only water microbiology but also the hygiene and sanitation practices of the depots. Of the 28 depots studied, 15 were reported as failing to meet eligibility requirements, while 13 met them. These findings indicate that hygiene and sanitation are critical aspects requiring attention in the management of refill drinking water depots.

Rezkina and Roslina (2024) reported a different finding. Their study examined *E. coli* and *Salmonella* sp. in refillable drinking water and found no growth of either organism in the samples examined. The difference between their findings and the present study indicates that microbiological quality may vary substantially

between locations. Therefore, negative findings in one geographic area cannot automatically be generalized to other drinking-water depots.

A recent study of refillable drinking-water depots in Padang City also provides a geographically relevant comparison. Rahayu *et al.,* reported that some of the examined depots did not comply with microbiological quality standards, with *E. coli* and total coliform detected in some samples. The researchers recommended improvements in cleanliness, sanitation, equipment maintenance, and routine microbiological testing. These findings are particularly relevant because Padang City is geographically close to Pariaman City and provides a useful regional comparison.

More recently, Savitri *et al.,* (2025) evaluated 22 refillable drinking-water depots in Kediri City. Their study found that 20 of 22 depots met the microbiological safety requirement of 0 CFU/100 mL for *E. coli*, while a smaller proportion did not meet the requirement. This finding demonstrates that many depots can achieve microbiological compliance, although some depots still require attention to microbiological safety.

Taken together, the findings of the present study are consistent with several previous studies demonstrating that *E. coli* is not necessarily detected in every refillable drinking-water sample, but contamination may still occur in a proportion of samples. Differences in contamination levels between studies may be related to sample size, geographic characteristics, source-water quality, treatment systems, sampling time, sanitation conditions, and analytical methods. Therefore, the present findings should be interpreted as a description of the examined samples rather than as a representation of all refillable drinking-water depots in Pariaman City.

Interpretation of the Findings Based on Scientific References

Escherichia coli is an important microbiological indicator because its presence in drinking water may indicate fecal contamination. The World Health Organization's *Guidelines for Drinking-water Quality* state that *E. coli* or thermotolerant coliform bacteria should not be detectable in any 100 mL sample of water directly intended for drinking. WHO also emphasizes that detection of *E. coli* should trigger investigation because it can indicate fecal contamination.

The detection of 13 CFU/100 mL in S.030a and 1 CFU/100 mL in S.031a demonstrates that both samples contained detectable *E. coli*. Indonesian Ministry of Health Regulation No. 2 of 2023 establishes a maximum allowable concentration of 0 CFU/100 mL for *E. coli* in drinking water. Therefore, both samples did not meet the microbiological requirement for this parameter. In contrast, the 0 CFU/100 mL results for S.032a and S.036a indicate that *E. coli* was not detected in the analyzed sample volume.

The variation among samples indicates that the microbiological quality of refillable drinking water was not uniform. Sample S.030a had a higher bacterial count than S.031a, whereas the other two samples showed no detectable *E. coli*. Such variation may be related to different aspects of the drinking-water production chain, including source-water quality, filtration, disinfection, storage conditions, equipment cleanliness, and handler hygiene. However, these factors were not directly evaluated in the present study and therefore cannot be identified as confirmed causes of contamination.

This interpretation is supported by Harianja *et al.,* (2022), who emphasized the importance of equipment maintenance and processing supervision in relation to *E. coli* contamination. Similarly, Julianti and Pawenang (2025) reported associations between premises sanitation, equipment sanitation, handler hygiene, and the presence of *E. coli* in drinking-water depots under health-office supervision in Rembang Regency. These findings suggest that sanitation management can be an important component of microbiological contamination control.

However, these findings do not establish that sanitation factors were responsible for the contamination observed in S.030a and S.031a. The present study did not conduct a systematic sanitation assessment, and therefore causal relationships cannot be established. Sanitation and depot-management factors should instead be considered potential variables for future investigation.

Microbiological safety may also change after water leaves the treatment process. Yunada *et al.,* (2023) investigated the microbiological

safety of refillable drinking water and changes during storage and highlighted the relevance of hygiene, sanitation, and storage duration in maintaining microbiological safety. This suggests that microbiological quality control should not stop at the treatment stage but should also include storage and handling conditions.

Overall, the detection of *E. coli* in two study samples indicates not only non-compliance with the applicable microbiological standard but also the need for continuous microbiological monitoring of refillable drinking water. The findings demonstrate that microbiological examination provides important information for drinking-water safety surveillance, particularly because refillable drinking water is commonly consumed directly by the public.

Implications and Follow-up of the Findings

The detection of *E. coli* in S.030a and S.031a indicates the need for follow-up actions, including repeat microbiological testing and evaluation of drinking-water management processes at the corresponding depots. Repeat testing is important to determine whether the positive findings represent persistent contamination or an isolated occurrence at the time of sampling. Periodic sampling can provide a more representative assessment of the consistency of microbiological quality. Indonesian Ministry of Health Regulation No. 2 of 2023 identifies *E. coli* as a mandatory drinking-water parameter and establishes a maximum allowable concentration of 0 CFU/100 mL. Therefore, positive findings should provide a basis for quality-control evaluation and corrective actions.

Follow-up evaluation may include assessment of the raw-water source, filtration system, disinfection process, storage conditions, filling equipment, and hygiene and sanitation practices. These assessments can help identify potential points at which contamination may occur. However, because these factors were not evaluated in the present study, further research is required before any specific contamination source can be identified.

Future research should also involve a larger number of samples and repeated sampling at different times. Repeated sampling would help determine whether contamination is temporary or persistent. Additional microbiological parameters, such as total coliforms and other relevant microorganisms, as well as selected physicochemical parameters, could also be incorporated to provide a more comprehensive assessment of drinking-water quality.

Follow-up actions may also involve strengthening monitoring by drinking-water depot operators and relevant authorities. Routine laboratory testing is important for verifying that produced drinking water continues to comply with microbiological requirements. WHO's updated drinking-water guidance emphasizes risk management from source to consumer, including surveillance and sanitary inspections.

Limitations of the Study

This study has several limitations that should be considered when interpreting the findings. First, the number of samples was relatively small, consisting of only four refillable drinking-water samples. Therefore, the findings cannot be generalized to represent all refillable drinking-water depots in Pariaman City. Rather, the results provide an initial description of the microbiological condition of the samples examined at the time of the study.

Second, the study focused only on *E. coli*. Therefore, the absence of detectable *E. coli* in S.032a and S.036a does not indicate that these samples were free from all microorganisms that may pose health concerns. Additional microbiological parameters would be required to provide a more comprehensive assessment of drinking-water quality.

Third, the study did not directly evaluate factors potentially associated with *E. coli* contamination, such as raw-water quality, filter condition, disinfection effectiveness, storage-tank condition, equipment cleanliness, and handler hygiene. Consequently, the study cannot determine the specific source of contamination.

Fourth, the samples were collected during a particular sampling period. Therefore, the findings represent the microbiological condition of the water at that specific time. Microbiological quality may change over time due to variations in treatment, storage, environmental conditions, and hygiene and sanitation practices. Future studies should therefore consider repeated sampling over different periods.

Another limitation is that the study focused on the culture-based detection and

enumeration of *E. coli*. Future studies may incorporate confirmatory or molecular approaches when more detailed characterization of the detected organisms is required. A 2024 study demonstrated that a molecular approach targeting *E. coli* could provide sensitive detection in drinking water and showed good agreement with the reference culture method.

Considering these limitations, the findings should be interpreted as an initial description of the microbiological condition of refillable drinking water in Pariaman City. Nevertheless, the detection of *E. coli* in two of four samples demonstrates the presence of a microbiological quality concern that warrants further examination and monitoring.

Conclusion

Microbiological quality assessments of drinking water samples in Pariaman City revealed deficiencies in the efficacy of the sterilization systems, as evidenced by the detection of *E. coli* bacteria in samples S.030a (13 CFU/mL) and S.031a (1 CFU/mL). Consequently, these samples were designated as Non-Compliant (*Tidak Memenuhi Syarat* / TMS) under the Ministry of Health Regulation (Permenkes) No. 2 of 2023. This contamination, detected via the membrane filter method, was triggered by UV system failures, the physical influence of water turbidity, and the presence of biofilms within the distribution lines. To consistently guarantee product safety for the public, comprehensive corrective actions are required, including routine maintenance of UV lamps and regular flushing of storage tanks to disrupt the bacterial growth cycle. Furthermore, strengthening operators' sanitary hygiene remains a crucial factor in preventing cross-contamination, thereby minimizing the risk of waterborne diseases as a form of sustainable public health protection.

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