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Comparison of the Microbiological Quality of RO and Mineral Refill Drinking Water Based on Presumptive Detection of *Escherichia coli*

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Abstract

The microbiological quality of refill drinking water is an important aspect in ensuring the safety of water consumed by the community. The presence of *Escherichia coli* can be used as an indicator of fecal contamination and is one of the important parameters in assessing the microbiological quality of drinking water. This study aims to compare the microbiological quality of reverse osmosis (RO) and mineral refill drinking water based on the presumptive detection of *Escherichia coli*. This study uses a descriptive observational design with a comparative approach based on laboratory examination. A total of 40 refill drinking water samples, consisting of 20 RO samples and 20 mineral samples from 20 depots, were examined at the Microbiology Laboratory of STIKES Papua from May to July 2026. Each depot contributed one RO sample and one mineral water sample. The examination was conducted through presumptive testing using Lactose Broth (LB), confirmatory testing using Brilliant Green Bile Broth (BGLB), and observation of colony characteristics on Eosin Methylene Blue (EMB) agar. The research results show that 8 out of 40 samples (20%) indicated presumptive positive results for *Escherichia coli*. In the RO group, 1 positive sample (5%) was found, while in the mineral water group, 7 positive samples (35%) were found. Analysis using the exact McNemar test showed a significant difference in the proportion of presumptive positive results between the two water groups (p = 0.031). Descriptively, the proportion of positive results was higher in the mineral water group compared to the RO group. These findings highlight the importance of monitoring the microbiological quality of refill drinking water and the implementation of good hygiene and sanitation practices in water management and filling. However, since the examination stopped at the observation of colony characteristics on EMB media, the positive results in this study are still presumptive and not yet a definitive confirmation of *Escherichia coli*. Therefore, further examination using biochemical tests or other validated identification methods is necessary to confirm the identity of the bacteria.

Article Information

Keywords: Refillable drinking water; *Escherichia coli*; Microbiological Quality; Reverse osmosis; presumptive

Kata kunci: minum isi ulang; *Escherichia coli*; Kualitas Mikrobiologis; Reverse osmosis; presumptif

Kualitas mikrobiologis air minum isi ulang merupakan aspek penting dalam menjamin keamanan air yang dikonsumsi masyarakat. Keberadaan Escherichia coli dapat digunakan sebagai indikator kontaminasi fekal dan menjadi salah satu parameter penting dalam penilaian kualitas mikrobiologis air minum. Penelitian ini bertujuan membandingkan kualitas mikrobiologis air minum isi ulang jenis reverse osmosis (RO) dan mineral berdasarkan deteksi presumtif Escherichia coli. Penelitian ini menggunakan desain observasional deskriptif dengan pendekatan komparatif berbasis pemeriksaan laboratorium. Sebanyak 40 sampel air minum isi ulang, yang terdiri atas 20 sampel RO dan 20 sampel mineral dari 20 depot, diperiksa di Laboratorium Mikrobiologi STIKES Papua pada Mei–Juli 2026. Setiap depot menyumbangkan satu sampel RO dan satu sampel air mineral. Pemeriksaan dilakukan melalui uji penduga menggunakan Lactose Broth (LB), uji penegasan menggunakan Brilliant Green Bile Broth (BGLB), dan pengamatan karakteristik koloni pada agar Eosin Methylene Blue (EMB). Hasil penelitian menunjukkan bahwa 8 dari 40 sampel (20%) menunjukkan hasil presumtif positif Escherichia coli. Pada kelompok RO, ditemukan 1 sampel positif (5%), sedangkan pada kelompok air mineral ditemukan 7 sampel positif (35%). Analisis menggunakan uji McNemar eksak menunjukkan adanya perbedaan proporsi hasil presumtif positif antara kedua kelompok air (p = 0,031). Secara deskriptif, proporsi hasil positif lebih tinggi pada kelompok air mineral dibandingkan dengan kelompok RO. Temuan ini menunjukkan pentingnya pengawasan kualitas mikrobiologis air minum isi ulang serta penerapan praktik higiene dan sanitasi yang baik dalam pengelolaan dan pengisian air. Namun, karena pemeriksaan berhenti pada pengamatan karakteristik koloni pada media EMB, hasil positif dalam penelitian ini masih bersifat presumtif dan belum merupakan konfirmasi definitif Escherichia coli. Oleh karena itu, pemeriksaan lanjutan menggunakan uji biokimia atau metode identifikasi tervalidasi lainnya diperlukan untuk memastikan identitas bakteri.

History

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A. INTRODUCTION

Safe drinking water is crucial for public health, and its microbiological quality is a key component of drinking water safety. *Escherichia coli* is widely used as an indicator of fecal contamination, and the World Health Organization recommends that this bacterium should not be detected in a 100 mL sample of drinking water intended for consumption (Organization, 2020). Previous studies in Indonesia have investigated the microbiological quality of refill drinking water and the presence of *Escherichia coli* in samples obtained from refill drinking water depots (Savitri et al., 2025); (Azteria & Rosya, 2023). However, these studies did not establish whether there is a difference in the detection of presumptive *Escherichia coli* between RO refill drinking water and mineral water collected from the same depot.

RO drinking water and mineral refill represent two categories of treatment available to consumers. Pairwise comparison of samples collected from the same depot is a way to evaluate differences in microbiological findings while considering the origin of the same depot. Therefore, this study aims to compare the proportion of presumptive positive *Escherichia coli* results in RO and mineral refill drinking water collected from 20 depots in the East Sorong District, Southwest Papua, Indonesia. We hypothesize that the proportion of presumptive-positive results will differ between the two categories of water. Depot sanitation, operator hygiene, and equipment condition were not directly assessed, therefore, this study did not evaluate the association of personal hygiene with the observed microbiological findings or establish it as a cause of differences between water categories. Additionally, since the examination was limited to presumptive testing and colony characteristics on eosin methylene blue (EMB) agar, positive findings were interpreted as presumptive identification rather than definitive identification of *Escherichia coli.*

In Indonesia, the microbiological quality of drinking water is regulated by Minister of Health Regulation No. 2 of 2023 on the Implementation of Government Regulation No. 66 of 2014 on Environmental Health. The regulation sets the maximum allowable level at 0 CFU/100 mL for *Escherichia coli* in drinking water (Indonesia, 2023). This requirement highlights the importance of microbiological monitoring to protect public health. In this study, however, the examination was limited to presumptive testing using Lactose Broth (LB), Brilliant Green Bile Broth (BGLB), and colony observation on Eosin Methylene Blue (EMB) agar. Therefore, the findings indicate a presumptive positive result rather than quantitative compliance with regulatory limits or definitive identification of *Escherichia coli.*

Refillable drinking water is widely used by the community because it is relatively easy to access and affordable. Its microbiological quality is an important consideration for drinking water safety. Several factors can affect the microbiological quality of refill drinking water, including the quality of raw water, the treatment process, and the effectiveness of filtration and disinfection systems (Arumsari et al., 2021). One of the treatment technologies used in the production of refill drinking water is reverse osmosis (RO), which separates water components through a semipermeable membrane. Differences in water treatment technologies may be related to variations in the microbiological characteristics of the final product (Bassam et al., 2026).

Microbiological examination for the detection of *Escherichia coli* may involve presumptive testing followed by confirmation and further identification procedures. In this study, Lactose Broth (LB) was used for the presumptive test, Brilliant Green Bile Broth (BGLB) for the confirmation test of coliform bacteria, and Eosin Methylene Blue (EMB) agar to observe colony characteristics indicating the presence of *Escherichia coli*. However, the colony characteristics on EMB agar alone are not sufficient for definitive species identification. (Souza et al., 2024) showed that the presence of a metallic green sheen on EMB agar does not reliably establish the identity of *Escherichia coli* in all cases and highlights the need for additional biochemical or molecular identification methods.

B. METHOD

This study uses a descriptive observational design with a comparative approach based on laboratory examinations to compare the microbiological quality of reverse osmosis (RO) drinking water and mineral refill based on presumptive detection of *Escherichia coli*. This study was conducted from May to July 2026. Water samples were taken from refill drinking water depots in the East Sorong District, Southwest Papua, Indonesia, and examined at the STIKES Papua Microbiology Laboratory. Purposive sampling was used to select 20 refill drinking water depots. The target population consists of refill drinking water depots operating in the East Sorong District. A total of 40 water samples were collected, consisting of 20 RO water samples and 20 mineral water samples. Each selected depot contributed one RO sample and one mineral water sample, resulting in 20 matched pairs. The total number of qualified depots in the district is not determined; therefore, the proportion of the depot population represented by the sample cannot be determined.

The sample size is determined pragmatically based on the research objectives, available resources, and research period. No formal statistical sample size calculations were performed. Each sample was collected once in a sterile container and labeled according to the depot code and its water category. No repeated sampling at different times was conducted. The inclusion criteria are that the depot is located in the East Sorong District, provides RO and mineral refill drinking water, and allows the collection of one sample from each type of water. Since both categories of water samples are taken from the same depot, the observations are treated as paired. Statistical independence is assumed between pairs from different depots, not between the two samples within each depot. Microbiological examination involves presumptive testing using Lactose Broth (LB), confirmation testing using Brilliant Green Bile Broth (BGLB), and observation of colony characteristics on Eosin Methylene Blue (EMB) agar. Because the examination does not include additional biochemical or molecular identification, positive findings are interpreted as the detection of suspected E. coli rather than definitive species identification.

The presumptive test was conducted using the double-tube fermentation technique with a Most Probable Number (MPN) configuration of 5:1:1. For each water sample, seven tubes containing Lactose Broth (LB) and inverted Durham tubes were prepared. Five tubes containing LB were each inoculated with 10 mL of the water sample. The remaining two tubes contained LB; one tube was inoculated with 1 mL of the sample, and the other with 0.1 mL. The tubes were incubated for 48 hours and examined for gas production in the Durham tube as well as changes in the liquid in the test tube. The tubes suspected to be positive were recorded for each inoculum volume and subsequently confirmed using Brilliant Green Lactose Bile Broth (BGLB). The MPN results are determined from the combination of positive tubes in each series using the appropriate 5:1:1 MPN table and reported as the MPN index per 100 mL of water, consisting of:

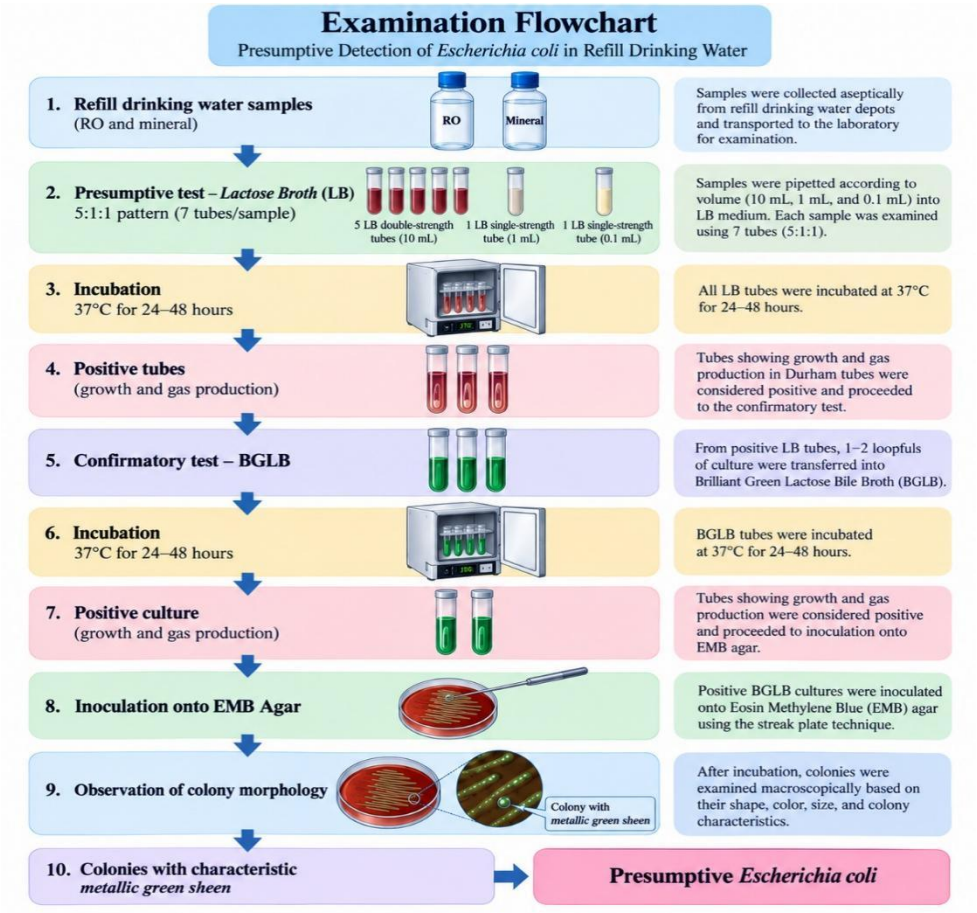
Table 1. Tube Methode Using the 5:1:1 Pattern

Number of Tubes	Sample Volume	Media
5	10 ml	LB
1	1 ml	LB
1	0,1 ml	LB
Total		**7 tube/sample**

The tube that shows a positive reaction at the LB stage is continued to BGLB, then the positive BGLB culture is inoculated onto EMB agar to observe the colony characteristics.

Microbiological examination was conducted using the double-tube fermentation procedure with a 5:1:1 tube configuration, followed by confirmation testing and observation of colony characteristics on Eosin Methylene Blue (EMB) agar. Sample collection and transportation. Water samples were collected in sterile sample containers, labeled with the appropriate depot code and water category, and transported to the STIKES Papua Microbiology Laboratory. The time interval between sample collection and laboratory examination is 30 minutes to 1 hour, and the transportation temperature and storage conditions are not measured. Presumptive test. Each water sample is homogenized before inoculation. Seven tubes containing Lactose Broth (LB) were prepared for each sample. Five tubes containing double-strength LB were each inoculated with 10 mL of water sample. One tube containing single-strength LB was inoculated with 1 mL of sample, and another tube containing single-strength LB was inoculated with 0.1 mL of sample. Each tube is equipped with an inverted Durham tube to detect gas production. After inoculation, the tubes were gently mixed and incubated at 37°C for 48 hours. After incubation, the tubes were examined for growth and gas production. The tubes that meet the presumptive-positive criteria are recorded for each inoculum volume. Confirmation test. The inoculum from each suspected positive LB tube is aseptically transferred into Brilliant Green Lactose Bile Broth (BGLB) using an inoculation loop. The inoculated BGLB tubes were incubated at 37°C for 48 hours and checked for gas production in Durham tubes. Positive tubes were recorded in Excel. Observation of colonies on EMB agar. Material from the BGLB tube that showed positive results was streaked onto EMB agar using the streak-plate technique. The plates were incubated at 37°C for 48 hours and examined macroscopically for colony growth, morphology, color, and other characteristic features. Colonies that exhibit a dark appearance with a metallic green sheen are recorded as presumptive *Escherichia coli*.

Image 1 Inspection flow



C. RESULT & DISCUSSION

A total of 40 refill drinking water samples were examined, consisting of 20 RO refill water samples and 20 mineral refill water samples. The examination was conducted in stages through presumptive tests using LB media, confirmatory tests using BGLB media, and observation of colony characteristics using Eosin Methylene Blue Agar (EMB) media

Tabel 2. Results of presumptive *Escherichia coli* detection in RO and mineral water based on the depot.

Depot ID	RO Water	Mineral Water
1	Negative	Negative
2	Negative	Positive
3	Negative	Positive
4	Negative	Negative
5	Negative	Positive
6	Negative	Negative
7	Negative	Negative
8	Negative	Negative
9	Negative	Positive
10	Negative	Negative
11	Negative	Negative
12	Negative	Negative
13	Negative	Negative
14	Negative	Negative
15	Negative	Positive
16	Negative	Negative
17	Negative	Negative
18	Negative	Positive
19	Positive	Positive
20	Negative	Negative

Table 2. Distribution of microbiological examination results based on water type and examination stages.

Water Type	Examination Stage	Positive, n(%)	Negative, n (%)	Total
RO	Presumptive Test(LB)	1 (5,0)	19 (95,0)	20
	Confirmatory Test (BGLB)	1 (5,0)	19 (95,0)	20
	EMB Agar	1 (5,0)	19 (95,0)	20
Mineral	Presumptive Test(LB)	7 (35,0)	13 (65,0)	20
	Confirmatory Test (BGLB)	7 (35,0)	13 (65,0)	20
	EMB Agar	7 (35,0)	13 (65,0)	20

Based on Table 2, out of 20 samples of RO refill drinking water, 1 sample (5.0%) showed a positive result in the presumptive test using LB. This sample remained positive in the confirmatory test using BGLB and produced colony characteristics that support the suspicion of *Escherichia coli* on EMB agar media. A total of 19 samples (95.0%) showed negative results at all stages of examination.

Among the 20 samples of mineral refill drinking water examined, 7 (35.0%) showed positive results in the presumptive test using Lactose Broth (LB). These seven samples also showed positive results in the confirmation test using Brilliant Green Lactose Bile Broth (BGLB) and exhibited colony characteristics indicating the presence of *Escherichia coli* on Eosin Methylene Blue (EMB) agar. However, these findings are considered presumptive because the colony morphology and the presence of a greenish metallic sheen on EMB agar are not sufficient to establish a definitive species identification. No additional biochemical or molecular identification tests were conducted. The remaining 13 samples (65.0%) showed negative results throughout the examination stages. Descriptively, the proportion of presumptive-positive results was higher in mineral refill drinking water compared to RO water (35.0% versus 5.0%, respectively).

Table 3. Comparison of final results of presumptive *Escherichia coli* detection based on water type

Water Type	Presumptif *Escherichia coli* positive, n(%)	Presumptif *Escherichia coli* negative, n(%)	Total
RO	1 (5,0)	19 (95,0)	20 (100)
Mineral	7 (35,0)	13 (65,0)	20(100)
Total	8 (20,0)	32 (80,0)	40(100)

Based on Table 3, out of 40 examined refillable drinking water samples, 8 samples (20.0%) showed presumptive positive for *Escherichia coli*, while 32 samples (80.0%) showed negative results. If categorized by water type, the RO group showed 1 positive sample out of 20 samples (5.0%) and 19 negative samples (95.0%). Meanwhile, the mineral water group showed 7 positive samples (35.0%) and 13 negative samples (65.0%). Thus, the proportion of presumptive positive *Escherichia coli* results in mineral water is 7 times higher proportionally compared to RO water, which is 35.0% compared to 5.0%. This finding is important from the perspective of drinking water safety because *Escherichia coli* is one of the main indicators of fecal contamination and its presence in drinking water. In the context of regulations in Indonesia, the management of Drinking Water Depots (DAM) currently refers to the applicable licensing provisions and business standards, including the Indonesian Minister of Health Regulation No. 11 of 2025. These regulations serve as one of the foundations for conducting business activities in the health sub-sector. From a microbiological perspective, the presence of *Escherichia coli* in drinking water is a finding that requires attention because water intended for consumption should not contain indicators of fecal contamination.

Table 4. Exact McNemar test results for paired data

Statistic	Result
Number of depot pairs	20
Statistical test	McNemar Exact
Two-sided p-value	0,031
Significanse level	0,05

Table 4 presents the results of the exact McNemar test comparing the paired proportions of suspected positive *Escherichia coli* results in RO and mineral refill drinking water samples taken from the same 20 depots. The exact two-sided p-value is 0.031, which is below the significance threshold of 0.05, indicating a statistically significant difference in the paired proportions. The findings show a difference in presumptive positive results between the two types of water but do not establish a causal relationship or confirm the definitive identity of *Escherichia coli*.

The microbiological quality of refill drinking water can be influenced by several factors, including the quality of raw water, the effectiveness of processing, the condition of filters or membranes, the cleanliness of storage tanks, the cleanliness of filling equipment, the sanitation of containers, and the cleanliness of operators. Previous studies have discussed the potential relevance of processing methods and sanitation practices on the microbiological quality of drinking water (Herwin et al., 2023; (Iqbal & Ane, 2022). Research in Indonesia has also reported a relationship between sanitation conditions, equipment cleanliness, and operator hygiene with the microbiological quality of refill drinking water (Julianti & Pawenang, 2024). The reverse osmosis system uses a semipermeable membrane that can reduce many contaminants, including microorganisms, when the system is operated and maintained properly. However, the performance of the membrane and the microbiological condition of the water after treatment depend on the overall treatment and handling process. In this study, membrane integrity, depot sanitation, operator cleanliness, and equipment cleanliness were not directly assessed. Therefore, these factors cannot be identified as explanations for the observed differences between RO water samples and mineral water. These findings actually support the importance of routine microbiological monitoring of refillable drinking water and further research into factors potentially related to contamination.

The findings of this study can be considered alongside the findings of (Abas et al., 2025), who reported *Escherichia coli* positive water samples in 30% of the refill drinking water depots studied in Mimika Regency. This study specifically compares the presumptive *Escherichia coli* positive results between RO and mineral refill drinking water samples taken from the same depot. The findings should also be interpreted in the context of the microbiological identification procedures used. Although positive results were observed at the LB and BGLB stages, and colony characteristics consistent with suspected *Escherichia coli* were observed on EMB agar, no further biochemical or molecular identification was performed. Therefore, the findings indicate the detection of suspected *Escherichia coli*. Further testing using validated biochemical or molecular methods is necessary to establish the identity of the suspected isolate.

This study is limited to 40 water samples taken from 20 depots in one district in Sorong City, Southwest Papua. Future studies should include more depots in various locations, use definitive bacterial identification methods, and directly assess the performance of the treatment systems as well as relevant hygiene and sanitation variables. These approaches will provide a more comprehensive understanding of the microbiological quality of RO drinking water and mineral refill in Southwest Papua.

D. CONCLUSION

A total of 40 samples of refill drinking water from 20 depots were examined for the initial detection of *Escherichia coli*. Of these, 8 samples (20.0%) showed presumptive positive results for Escherichia coli. The proportion of presumptive-positive results was 5.0% (1/20) for RO water and 35.0% (7/20) for mineral water. Although the proportion was higher for mineral water, the comparison was made using the exact McNemar test because each depot contributed one sample of each type of water. As shown by the paired results, one depot produced presumptive positive results for both RO water and mineral water, six depots produced positive results only for mineral water, no depot produced positive results only for RO water, and 13 depots produced negative results for both types of water. The exact McNemar test showed a statistically significant difference in the paired proportions of presumptive-positive results between RO water and mineral water ($p = 0.031$, two-sided). These findings indicate that the observed presumptive-positive results differ significantly between the two categories of water sampled at the depot. However, the results do not establish a causal relationship between the type of water and bacterial detection. Furthermore, since no biochemical or molecular identification

was performed after the EMB examination, the findings should be interpreted as a detection of suspected definitive identification of *E. coli*.

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