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Assessment of *Escherichia coli* Contamination in Drinking Water from Refill Depots

Lisa Savitri[1,3,4,*], Muhammad Akbar Nur Kholis[1], Fendy Prasetyawan[2], Yuneka Saristiana[2], Cornelia Amanda[1], Konradus Klala Mebung[1]

[1]Department of Medical Laboratory Technology, Faculty of Health Sciences, Kadiri University, Jalan Selomangleng No. 1, Kediri, East Java, Indonesia
[2]Department of Pharmacist Professional Education, Faculty of Health Sciences, Kadiri University, Jalan Selomangleng No. 1, Kediri, East Java, Indonesia
[3]Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia
[4]Bioinformatics Research Center, Indonesian Institute of Bioinformatics, Malang, Indonesia.

Corresponding author*
lisasavitri@unik-kediri.ac.id

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Abstract

Refill Drinking Water Depots (DAMIU) have become a popular source of drinking water in Indonesia due to their affordability and accessibility. However, concerns about hygiene and microbial contamination persist, particularly in areas with limited health oversight. This study aimed to evaluate the microbiological quality of refilled drinking water in Kediri City by detecting the presence of *Escherichia coli* as an indicator of fecal contamination. A descriptive, cross-sectional design involving 22 depots selected based on hygiene criteria was employed. Microbiological analysis used the Most Probable Number (MPN) method, with confirmatory tests including IMViC and Eosin Methylene Blue (EMB) media. The results showed that 20 of 22 depots met the microbiological safety standard of 0 CFU/100 mL for *E. coli* as set by the Indonesian Ministry of Health and national standards (SNI 3554:2015). Two samples (5 and 7) tested positive for *E. coli*, likely due to environmental contamination, inadequate sanitation, and poor water handling practices. The positive samples demonstrated typical *E. coli* characteristics in IMViC (++-+) and gas formation in BGLB media. This study underscores the importance of routine surveillance, public health regulation enforcement, and improved hygiene practices in refilled water depots. Ensuring the microbiological safety of refilled drinking water is essential to prevent waterborne diseases such as diarrhea. The findings call for enhanced collaboration between local health offices and depot operators to maintain clean and safe drinking water standards across communities.

Keywords: Refill Drinking Water Depot; *Escherichia coli*; Water Quality; MPN Method; Public Health.

Abbreviations: Brilliant Green Lactose Broth (BGLB), Badan Pengawas Obat dan Makanan (National Agency of Drug and Food Control) (BPOM), Colony Forming Unit (CFU), Depot Air Minum Isi Ulang (Refill Drinking Water Depot), (DAMIU), Eosin Methylene Blue (EMB), Inspeksi Kesehatan Lingkungan (Environmental Health Inspection) (IKL), Higiene Sanitasi Pangan (Food Sanitation Hygiene) (HSP), Indole, Methyl Red, Voges-Proskauer, Citrate (IMViC), Lactose Broth (LB), Most Probable Number (MPN), Sertifikat Laik Higiene dan Sanitasi (Hygienic and Sanitary Feasibility Certificate) (SLHS), Standar Nasional Indonesia (Indonesian National Standard) (SNI)

INTRODUCTION

Refill Drinking Water Depots (DAMIU) are businesses engaged in drinking water management. The public often uses refill depots due to their affordability and accessibility. This type of business has grown rapidly across Indonesia, including in remote areas with limited access to clean water. According to a study by Zikra et al. (2018), the daily water sources used by Indonesians include dug wells (24.7%), piped water (14.2%), boreholes (14.0%), and refill drinking water depots (13.8%). Drinking water is defined as water, either treated or untreated, that is safe for direct consumption and meets health standards.

Efforts to improve water quality involve monitoring, protection, and enhancement, conducted by various parties including the government and relevant institutions such as the Ministry of Industry. Surveillance and quality inspections are carried out nationwide, including at refill depots. Based on 2023 data from the Ministry of Industry, approximately 31.87% of Indonesia's population relies on refilled water as their primary drinking water source. By the first quarter of 2024, there were 78,378 refill depots in Indonesia, of which only 53,261 met Food Sanitation Hygiene (HSP) standards, and only 1,755 had received a Hygienic and Sanitary Feasibility Certificate (SLHS). Water quality monitoring involves surveillance, laboratory testing, risk analysis, and follow-up recommendations.

Most refill depots are managed individually as micro, small, and medium enterprises (MSMEs). A study conducted by Mirasa included 25 regencies and cities out of 38 in East Java Province, analyzing 1,113 water samples from DAMIU. The sampling method followed the Indonesian National Standard SNI 7828:2012 (Mirasa et al., 2024). In Kediri City, 26 refill depots providing purified water at prices aligned with the purchasing power of lower-middle-income groups, making refill depots a preferred choice (Efendi, 2019). However, affordable prices must not compromise quality—every business must establish standards that satisfy consumers and enhance competitiveness. With increasing business competition, companies must develop appropriate strategies to adapt to future economic changes and stay competitive in the same sector (Eka, 2023). High-quality water can suppress microbial growth and reduce the presence of harmful microorganisms in refill depots (Kiman et al., 2019).

Microbial growth in water is a biological parameter used to assess water cleanliness. One of the key groups evaluated is coliform bacteria, characterized as rod-shaped, gram-negative, non-spore-forming, and capable of living in aerobic and facultatively anaerobic conditions. Coliforms, including fecal and non-fecal subgroups, naturally inhabit the intestines of humans and animals. These bacteria ferment lactose, producing acid and gas within 48 hours. Some strains produce harmful substances such as ethionine, indole, and skatole, which are carcinogenic and toxic when present in significant amounts.

The presence of coliform bacteria indicates fecal contamination from humans or animals and the potential presence of other pathogenic bacteria. It also suggests a risk of dangerous enteropathogenic and toxigenic microbes in the water. Humans are at risk of diseases like diarrhea from consuming water contaminated with coliforms and *E. coli*. Although *E. coli* is a normal part of intestinal flora in both humans and animals, it can become pathogenic when present outside the digestive tract (Hidayati et al., 2018). *E. coli* and coliforms are key indicators of water contamination and help assess the potential presence of harmful microbes. Outside the gastrointestinal tract, *E. coli* can cause diseases such as diarrhea (Afriyanti, 2019).

One of the leading causes of diarrhea is consuming drinking water contaminated with bacteria like *E. coli*. People consuming water from wells, rivers, piped water, bottled water, or refill depots may be at risk. Ready-to-drink water is often stored before consumption, and its quality can deteriorate during storage or distribution. Ensuring water quality is crucial to public health and preventing microbial proliferation. Microorganism presence is a major determinant of water quality, and excessive amounts can lead to diseases such as diarrhea.

Drinking water must comply with health standards and be free from coliform bacteria. According to Indonesia's Ministry of Health Regulation No. 2 of 2023, the acceptable level of coliform bacteria is 0/100 mL. Many refill depots fail to meet this requirement due to poor hygiene practices, improper handling, and insufficient cleaning. Bacteria can grow on improperly maintained equipment and containers, resulting in unsafe water.

Preliminary observations and environmental health inspection data (IKL) from Campurejo Public Health Center in Kediri revealed 14 refill depots, most of which used groundwater as a source. These depots offer affordable water with delivery services, making them attractive to consumers. However, concerns remain about the hygiene and sanitation conditions of the refill depots. Risks arise due to depot locations, packaging practices using reusable plastic gallons, and lack of knowledge among operators about hygiene and sanitation. Many depots are located near dusty roads, and employees often do not wear proper uniforms or head coverings. Some depots a lack adequate handwashing stations and covered waste bins (Hartanto et al., 2022).

MATERIALS AND METHODS

This study employed a descriptive design using a quantitative, cross-sectional, and laboratory-based experimental approach. The research was conducted on 22 refilled drinking water depots in Kediri City. Initial observations were made to assess hygiene-related factors, followed by microbiological testing using the Most Probable Number (MPN) method, which included presumptive tests with Lactose Broth (LB) medium and confirmatory tests using Eosin Methylene Blue (EMB) agar. The study population comprised all refill drinking water depots (DAMIU) in Kediri City, while the samples were selected based on inclusion criteria such as being operational, willing to participate, and considered to have poor hygiene. Depots without supervision from the local health office were excluded.

The research involved three variables: the independent variable was the refill water depot, the dependent variable was the presence of *E. coli*, and the control variables included incubation temperature, media composition, and duration. Operational definitions were used to measure the variables, and results were categorized based on standard criteria. Materials used in this study included water samples, LB, EMB, BGLB, MR-VP, and INDOL media, mostly from Himedia. Instruments included test tubes (Iwaki), micropipettes, incubators, and autoclaves.

Media preparation involved weighing and dissolving reagents followed by sterilization. Microbial inoculation was performed using serial dilution in LB, followed by incubation, confirmed testing in BGLB, and completed testing using EMB, INDOL, MR-VP, and citrate media. Data were presented in frequency tables to assess the

presence of *E. coli* contamination in the refilled drinking water.

RESULTS AND DISCUSSION

Results

Table 1. Results of Presumptive Test on Refilled Drinking Water Using BGLB Media.

Sample	5 x 10 mL Tubes	1 x 10 mL Tube	0.1 x 10 mL Tube	MPN Index /100 mL
1	0	0	0	0/100 mL
2	0	0	0	0/100 mL
3	0	0	0	0/100 mL
4	0	0	0	0/100 mL
5	1	0	0	2.2/100 mL
6	0	0	0	0/100 mL
7	1	0	0	2.2/100 mL
8–20	0	0	0	0/100 mL
Positive Control	5	1	1	240/100 mL
Negative Control	0	0	0	0/100 mL

Out of the 22 depot water samples tested, only 2 samples did not meet the quality standards, while 20 samples met the requirements. According to BPOM and SNI 3554, the maximum microbial contamination limit for *E. coli* is undetectable at 0/250 mL. In this study, *E. coli* was detected in samples No. 5 and 7, while it was not detected in samples No. 1–4, 6, and 8–20. The study used mineral water and river water as positive and negative controls.

Table 2. Research Results on IMViC & EMB Media.

Sample No.	Indole	VP	MR	Citrate	EMB	Remarks
5	+	+	–	+	+	*E. coli* (+)
7	+	+	–	+	+	*E. coli* (+)
Control (+)	+	+	–	+	+	*E. coli* (+)

According to Table 5.2, both samples showed positive reactions on IMViC (++-+) and gas formation in the BGLB medium, confirming the presence of *E. coli*. These results are consistent with the positive control (river water). Gede et al. (2023) stated that IMViC testing is performed on samples suspected of contamination, confirming Gram-negative bacilli by Gram staining and metallic sheen colonies on EMB agar.

Discussion

The study revealed that 2 out of 22 samples tested positive for *E. coli*, specifically samples 5 and 7, with values >0 CFU/100 mL. This contamination likely resulted from domestic wastewater near the water sources, including dishwashing, laundry, and bathing. According to the Ministry of Health Regulation No. 2 of 2023, hygiene and sanitation aim to ensure drinking water is free from microbial, physical, chemical, and radioactive contaminants. The test results complied with BPOM and SNI 3554 standards, with *E. coli* undetected in 18 samples (0/250 mL), indicating most depot waters in Kediri city meet the standards and can reduce diarrhea cases caused by contaminated refilled water.

Based on the results in Tables 1 and 2, although the overall water quality met drinking water standards, samples 5 and 7 exceeded the permissible limits. These refilled waters were prone to contamination due to their roadside locations, which made them susceptible to airborne dust and pollutants. Previous research by Winarti (2019) in Palembang reported a high rate of positive *E. coli* samples, underscoring the need to tighten public health regulations.

MPN tests showed the presence of coliform and *E. coli* bacteria in the two contaminated samples. This aligns with findings by Retno (2019), where only 2 out of 22 (9.1%) depots in the Ngasem area, Kediri, did not meet Ministry of Health standards. Neli Zulfa et al. (2023) found that 25% of water depots contaminated with *E. coli*, 11 with coliform bacteria, and 3 with pH values outside acceptable limits of 28 supplemental drinking water storage facilities, 15 (53.6%) did not meet quality standards, while only 13 (46.6%) were compliant. Yellow tubes indicated positive *E. coli* results (2.2/250 mL), and blue tubes indicated gas presence in BGLB.

According to Hartanto et al. (2022), 78.57% of depots in the Campurejo Health Center area, Kediri, met microbiological water quality standards, while 21.43% did not, as per Health Minister Decree No. 492/MENKES/PER/IV/2010. Improved standards could help reduce diarrhea incidence from depot-sourced drinking water.

Positive MPN results were indicated by gas formation in Durham tubes using Lactose Broth media. Only two out of twenty samples contained *E. coli*. These findings suggest that although sanitation and hygiene practices in Kediri have improved, there is still inadequate supervision by local health authorities. Some operators disregard drinking water quality. Water quality in depots depends on raw water sources, sanitation of workers, handling of containers by consumers, and storage conditions.

Confirmed tests were conducted by transferring gas-positive BGLB tubes into new BGLB media and incubating at 36°C for 24–48 hours. Turbidity and gas produstion indicated a positive result. In Table 5.2, samples 5 and 7 were confirmed positive at the 5 x 10 mL dilution level. Gas formation resulted from lactose fermentation by coliform bacteria.

Brilliant Green Lactose Broth (BGLB) supports the growth of Gram-negative, lactose-fermenting coliforms, while inhibiting Gram-positive bacteria. Coliform bacteria produce energy through lactose fermentation,

generating pyruvate, acetate, and CO_2, which forms visible gas bubbles in the Durham tubes (Putri et al., 2018). Higher MPN values indicate greater microbial contamination, while lower values reflect better water quality.

CONCLUSIONS

This study evaluated the microbiological quality of refilled drinking water from 22 depots (DAMIU) in Kediri City, Indonesia, focusing on the presence of Escherichia coli as an indicator of fecal contamination. The findings revealed that 90.9% (20 out of 22) of the depots met the microbiological standards set by BPOM and SNI 3554, while 9.1% (samples 5 and 7) exceeded the acceptable limit of 0 CFU/100 mL for E. coli. Confirmatory testing through IMViC and EMB media supported the presence of E. coli in these two samples. The contamination in these cases may be attributed to inadequate sanitation, poor hygiene practices, improper handling of containers, and depot locations near pollution sources. Despite improvements in hygiene across the sector, the study highlights the need for stricter supervision, increased awareness among depot operators, and enforcement of health regulations to ensure consistent water quality. As refill water remains a primary source of drinking water for many Indonesians, ensuring its safety is essential to protect public health and prevent waterborne diseases such as diarrhea. Future initiatives should focus on strengthening health inspections, enhancing operator training, promoting better sanitation infrastructure, and increasing public education on safe water handling practices.

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