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RESEARCH ARTICLE

Contamination with *Escherichia coli* in jumbo iced tea sold on Ayahanda Street, Medan: A Most Probable Number (MPN) approach

Santa Ronauli Matondang[1], Edy Fachrial[2,3]*, Wienaldi[2,4], Joshua Asley[3,5], Nyi Raden Siti Vissy Puspa Dewi Muharam[3,5], Intan Aryanti[3,5], Nicolas Xavier Ongko[3,5], Sahna Ferdinand[3,5], Tri Amelia Sari[3,5]

ABSTRACT

Background: Diarrheal diseases are primarily transmitted through contaminated food and water, including ready to consume beverages like jumbo iced tea commonly sold by street vendors in urban areas. These beverages are highly susceptible to environmental contamination due to open air preparation and serving. This study aims to identify and quantify coliform bacteria in jumbo iced tea sold on Ayahanda Street, Medan.
Method: A descriptive quantitative cross-sectional design was employed, analyzing five samples selected via purposive sampling in February 2026. The microbiological contamination was evaluated using the Most Probable Number (MPN) approach, which consisted of presumptive, confirmed, and completed tests.
Results: The results revealed that all samples tested positive for coliform bacteria, presenting MPN values ranging from 240 to greater than 1100 MPN/100 mL. These findings completely violate the microbiological quality standards for safe drinking water, which strictly require 0 MPN/100 mL. Furthermore, isolation on Eosin Methylene Blue (EMB) agar exhibited metallic green colonies, confirming the specific presence of *Escherichia coli* in all tested beverages.
Conclusion: In conclusion, jumbo iced tea sold in this specific region is heavily contaminated with fecal coliforms, rendering it entirely unfit for human consumption. These findings highlight the critical necessity for strict implementation of food sanitation and hygiene practices during food processing and serving to mitigate severe public health risks.

Keywords: jumbo iced tea, coliform, Escherichia coli, MPN approach, sanitation hygiene

Introduction

Diarrheal disease is largely transmitted through contaminated food and water. The World Health Organization reports that foodborne illnesses cause approximately 600 million cases, 420,000 deaths, and 33 million disability-adjusted life years (DALYs) annually, with children under five accounting for 125,000 deaths each year. Children are disproportionately affected, as nearly 30% of all foodborne disease deaths occur in this age group despite representing only 9% of the global population.[1,2] In Indonesia, diarrhea remains endemic and can produce outbreaks, contributing substantially to the persistently high mortality rate among children under five.[3,4]

Affiliation
[1]Undergraduate Program in Medicine, Universitas Prima Indonesia
[2]Department of Biochemical, Universitas Prima Indonesia
[3]PUI Phyto Degenerative & Lifestyle Medicine, Universitas Prima Indonesia
[4]Department of Family Medicine, Universitas Prima Indonesia
[5]Department of Clinical Medicine, Universitas Prima Indonesia

Corespondence:
edyfachrial@unprimdn.ac.id

National statistics show a diarrhea prevalence of 6.8% in 2018, an increase from 4.6% in 2013. Age distribution of cases in Indonesia is dominated by children aged one to four years, with the highest prevalence at 11.5%, followed by infants under one year at 9.0%, individuals aged 15 to 24 years at 6.7%, and children aged five to 14 years at 6.2%. At the regional level, the Central Bureau of Statistics for North Sumatra Province reported 70,243 diarrhea cases across all age groups in 2020. Medan ranked second in case counts with 8,047 reports, after Deli Serdang Regency, which reported 15,185 cases.[3,5]

High incidence is closely associated with exposure to street foods and beverages, particularly among children, who are the most frequent consumers. Children tend to prefer low-cost, easily accessible foods with appealing flavors.[6,7] This pattern is evident in Medan, especially along Jalan Ayahanda, a densely populated area with substantial street food vending activity. Street-vended beverages are particularly concerning because improper handling, poor hygienic practices, and lack of environmental control significantly increase contamination risk.

One commonly sold item by vendors in this area is "es teh jumbo" (large sweet iced tea). Vendors typically serve this beverage outdoors, which increases the risk of contamination from dust, air pollution, and potentially nonpotable water or ice. Studies on street-vended beverages have found that approximately 71% of samples were contaminated with total coliforms, with average readings ranging between 4.30 and 4.75 log CFU/mL. Because children's immune systems are not fully developed, consuming beverages that may be contaminated with coliform bacteria can raise the risk of gastrointestinal illnesses, including diarrhea.

Therefore, investigating the presence of coliform bacteria in es teh jumbo sold along Jalan Ayahanda is urgently needed to assess product safety and protect child consumers. The study's findings are expected to contribute to the scientific literature on food microbiology, specifically regarding detection of coliform contamination in ready-to-drink beverages, an area where recent research has identified significant microbiological safety gaps. The results should also serve as a reference for students and researchers conducting future studies on the microbiological safety of consumer beverages, supporting evidence-based interventions for improving food safety systems.

Method

This study used a quantitative descriptive approach with a cross-sectional design to evaluate coliform bacterial contamination in jumbo iced tea products. Data collection occurred in February 2026. The study population comprised all jumbo iced tea sold by street vendors along Jalan Ayahanda. From this population, five beverage samples were collected using purposive sampling based on predefined criteria. The sample size was chosen because of operational time constraints and to serve as a preliminary study to obtain an initial estimate of microbiological contamination in the study area.

Sample selection followed specific inclusion and exclusion criteria to ensure test accuracy. Inclusion criteria were jumbo iced tea sold by street vendors at the study location, presented ready-to-drink without reheating, and served with ice cubes. Sampling was required to occur at a consistent time, midday, to avoid contamination variability due to differences in outdoor exposure time. Exclusion criteria eliminated beverages served without ice, products not ready for consumption or still in preparation, and samples accidentally contaminated during non-aseptic collection. Products from vendors who declined participation and beverages displayed or stored for more than approximately four hours after initial serving were also excluded from analysis.

The independent variable was the water source, defined as the type of water used to prepare the iced tea, measured on a nominal scale. The dependent variable was coliform bacterial count, measured in the laboratory on a ratio scale. The primary data collection instrument was the Most Probable Number method, implemented with laboratory equipment and the following media: Lactose Broth, Brilliant Green Lactose Bile Broth, and Eosin Methylene Blue agar. Presence of coliform bacteria was validated and recorded on a nominal scale by observing gas formation in Durham tubes during testing; gas production served as the principal indicator to confirm the reliability of microbiological findings.

Laboratory procedures followed systematic microbiological testing based on coliform bacteria producing gas during incubation. Incubation targeted a temperature of approximately 36 degrees Celsius. The first stage was a presumptive test that used nine sterile test tubes containing lactose broth and a Durham tube. The tubes were arranged in three dilution series with volumes of 10.0 milliliters, 1.0 milliliter, and 0.1 milliliter, prepared using 0.9 percent sodium chloride solution as diluent. All tubes were incubated aseptically

at 37 degrees Celsius for two successive 24-hour periods. Tubes showing gas production were considered presumptively positive and proceeded to the next stage.

In the second stage, a confirmatory test was performed by inoculating material from positive presumptive tubes into Brilliant Green Lactose Bile Broth and incubating at 37 degrees Celsius for 24 hours. A positive result in this phase was indicated by media turbidity and gas production, consistent with coliform presence. The third stage, a completed test, involved transferring material from positive tubes onto Eosin Methylene Blue agar and incubating again at 37 degrees Celsius for 24 hours. *Escherichia coli* colonies were identified by their characteristic metallic green sheen, while non-fecal coliform colonies produced a pink coloration.

Data analysis consisted of observing physical changes in sample tubes after the full incubation period. Samples were classified as positive for coliform contamination if turbidity or gas bubbles were observed in the Durham tube. Each positive tube result was matched to a standard table to obtain a quantitative estimate of bacterial density according to standard microbiological examination procedures. The final step of descriptive statistical analysis summarized the number of samples containing coliform bacteria compared with samples free of such contamination.

Results

LB medium was used to perform presumptive testing. Samples were considered positive if gas bubbles appeared in the Durham tube. The presence of these bubbles in LB indicates that coliform bacteria are fermenting lactose. Results from the presumptive LB test showing gas production in Durham tubes are shown in Figure 1. The MPN-based coliform counts from the presumptive test are presented in Table 1.

Figure 1. Presumptive test results.

Table 1. Identification of coliform counts from the presumptive test

Sample	1 mL (3 tubes)	0.1 mL (3 tubes)	0.01 mL (3 tubes)	MPN/100 mL	Conclusion	Note
XY1	3	3	0	240	Positive	Does not meet standard
XY2	3	3	3	>1100	Positive	Does not meet standard
XY3	1	0	0	3.6	Positive	Does not meet standard
XY4	3	3	3	>1100	Positive	Does not meet standard
XY5	3	3	3	>1100	Positive	Does not meet standard

All samples produced gas in the Durham tubes during the LB presumptive test, indicating the presence of coliform bacteria capable of fermenting lactose and producing gas. MPN values from the presumptive test varied among samples. Samples XY2, XY4, and XY5 had MPN values >1100 MPN/100 mL, indicating very high contamination. Sample XY1 had an MPN of 240 MPN/100 mL, which also indicates substantial coliform contamination. Sample XY3 had an MPN of 3.6 MPN/100 mL; although lower than the others, it remained positive for coliforms. Variation in MPN across samples indicates nonuniform microbiological contamination, which may reflect differences in water quality, ice hygiene, equipment condition, or food-handler hygiene. A greater number of positive tubes at each dilution corresponds to a higher calculated MPN.

Selective coliform testing was performed to validate that the organisms detected in the presumptive test were coliforms. As an initial screen, the presumptive test indicated that all jumbo iced tea samples contained coliform bacteria.

Because coliforms were detected in all examined samples, the study proceeded to confirmation testing. Inoculation of positive samples into BGLB medium allowed confirmation testing. A successful confirmation was recorded when gas and turbidity were present in the Durham tube. BGLB is selective because it promotes coliform growth while inhibiting Gram-positive bacteria. Confirmation results showing gas production in Durham tubes on BGLB are shown in Figure 2. The MPN-based coliform counts from the confirmation test are shown in Table 2.

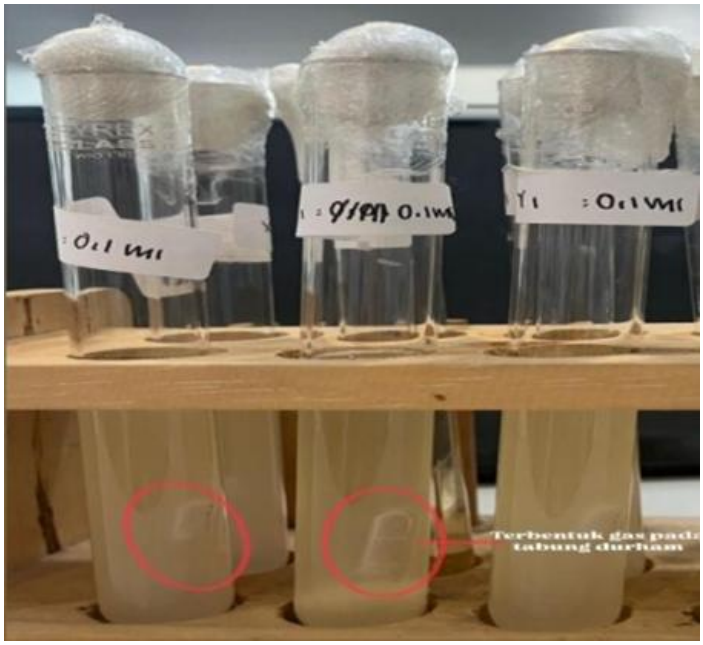

Figure 2. Confirmation test results on BGLB showing positive tubes (turbidity and gas bubbles)

Table 2. Identification of coliform counts from the confirmation test

Sample	1 mL (3 tubes)	0.1 mL (3 tubes)	0.01 mL (3 tubes)	MPN/100 mL	Conclusion	Note
XY1	3	3	3	>1100	Positive	Does not meet standard
XY2	2	3	3	1100	Positive	Does not meet standard
XY3	3	3	0	240	Positive	Does not meet standard
XY4	3	3	3	>1100	Positive	Does not meet standard
XY5	3	3	3	>1100	Positive	Does not meet standard

All jumbo iced tea samples (XY1–XY5) were positive for coliforms on confirmation testing, with MPN values exceeding acceptable limits. Sample XY1 showed a 3–3–3 tube pattern across dilutions (1 mL, 0.1 mL, 0.01 mL) corresponding to MPN >1100 MPN/100 mL, indicating that coliforms were detectable even at the highest dilution and reflecting very high contamination. The large number of positive tubes across dilutions indicates substantial bacterial counts in the sample. Sample XY2 had a 2–3–3 pattern with an MPN of 1100 MPN/100 mL; although fewer tubes were positive at the highest concentration than in XY1, the MPN remains very high and far above microbiological quality standards. These results indicate persistent contamination across dilution levels.

Sample XY3 showed a 3–3–0 pattern with an MPN of 240 MPN/100 mL. This value is lower than for other samples but still indicates significant contamination because it exceeds the maximum allowable limit of 0 MPN/100 mL. The absence of gas at the highest dilution suggests a relatively lower bacterial load compared with other samples, yet it still fails safety standards. Samples XY4 and XY5 matched XY1 (3–3–3; MPN >1100 MPN/100 mL), indicating very high contamination and suggesting a consistent contamination source, such as inadequate water or ice quality. Overall, all samples had MPN values above the microbiological quality limit for ready-to-drink beverages (0 MPN/100 mL).

E. coli identification was performed by inoculating positive confirmation-test isolates onto Eosin Methylene Blue Agar (EMBA). The presence of metallic green colonies indicates contamination by Escherichia coli. Therefore, isolates producing this colony morphology were identified as E. coli. Purple-pink colonies on EMBA indicate the growth of other coliform species.

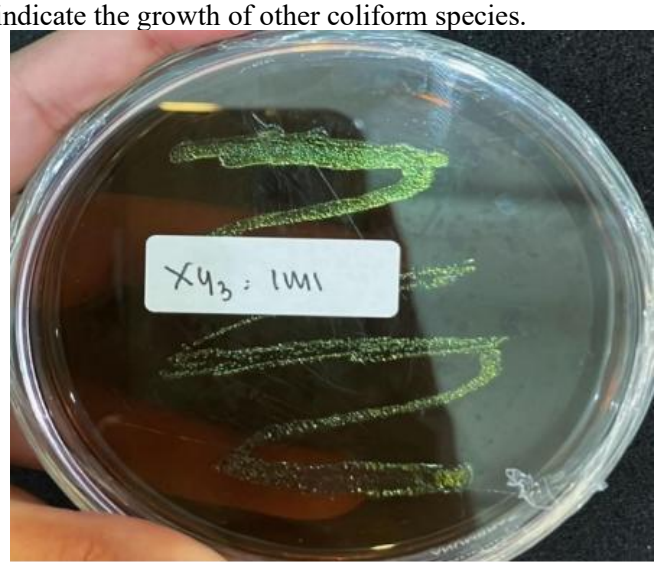

Figure 3 Identification of *Escherichia coli* on EMBA, showing characteristic metallic green colonies.

Figure 3 shows EMBA results with characteristic metallic green colonies of Escherichia coli. *E. coli* ferments lactose strongly, producing high acid levels that react with eosin and methylene blue indicators in the medium, yielding metallic green colonies. All jumbo iced tea samples were contaminated with fecal coliforms, particularly Escherichia coli, and did not meet microbiological quality standards for ready-to-drink beverages.

Discussion

The primary findings of this investigation reveal a severe level of microbiological contamination in jumbo iced tea beverages sold along Ayahanda Street, as evidenced by Most Probable Number values ranging from 240 to exceeding 1100 per 100 milliliters. These results unequivocally demonstrate that the tested samples grossly violate the established safety thresholds for ready to consume beverages, which mandate a strict zero tolerance for coliform bacteria. The isolation of metallic green colonies on Eosin Methylene Blue agar further confirms the definitive presence of Escherichia coli, serving as a direct bioindicator of recent fecal contamination. This profound level of bacterial presence strongly suggests severe inadequacies in the hygiene practices of street vendors, likely stemming from the use of contaminated raw water, unsanitary ice blocks, or inadequate equipment sterilization. Consequently, these findings highlight a critical vulnerability in the local informal food sector, where open air preparation and prolonged exposure to environmental pollutants severely compromise the microbiological integrity of popular consumer beverages.

These alarming findings are heavily corroborated by a robust body of recent literature emphasizing the pervasive microbiological risks associated with street vended beverages in developing regions. For instance, research on street food vendors in Southeast Asia demonstrated that most still use bucket water (non-tap water) rather than treated municipal water for beverage preparation and cleaning, creating significant hygiene vulnerabilities. Similarly, investigations into Indonesian aquatic environments have confirmed that local water bodies act as significant reservoirs for *Escherichia coli* pathogens, which inevitably cross-contaminate the food supply chain when untreated water is used for dishwashing or ice production.[8,9] Additional studies focusing on street foods in Makassar reported coliform contamination levels exceeding 1,100 Coliform/g in snacks like siomay and meatballs, far above the national standard limit of 10 Coliform MPN/g.[10] Furthermore, recent global meta-analysis indicates that 62% of hands in low-income countries are contaminated with *E. coli*, with hand hygiene deficiencies and absence of basic sanitary infrastructure at street stalls identified as primary vectors for introducing fecal bacteria into ready-to-drink liquids.[11] A 2019 study of street-vended beverages in Kuala Lumpur similarly found average safety levels requiring improvement, with improper handling and poor hygienic practices affecting beverage safety.[12]

Conversely, the extreme contamination levels observed in this study contrast sharply with microbiological profiles of beverages prepared in strictly regulated or indoor food service establishments. Recent global analyses of frozen and iced beverage safety incidents (2015–2024) show that franchised indoor vendors typically report total coliform counts within safe consumption limits, largely due to mandatory use of ultraviolet (UV) sterilized water and automated ice machines that significantly reduce microbial contamination. UV-C disinfection technology has been widely validated for inactivating *Escherichia coli* and other pathogens in food and beverage operations, meeting USDA and FSIS sanitary safeguards.[13,14]

Furthermore, while this study found ubiquitous *E. coli* presence, research from developing urban centers demonstrates that targeted vendor education programs combined with provision of potable water stations can drastically reduce coliform prevalence in street-vended foods to near-undetectable levels. This discrepancy indicates that contamination is not an inherent characteristic of iced tea itself but is entirely conditional upon the socioeconomic and infrastructural environments where preparation occurs.[15] Additionally, some scholars note that coliform counts alone may occasionally overestimate public health risks due to environmental non-fecal coliform strains. However, the specific isolation of *Escherichia coli* in this study confirms a definitive fecal origin, as *E. coli* is a well-established fecal indicator organism distinct from general environmental coliforms.[16]

Conclusion

The findings indicate that jumbo iced tea beverages sold by street vendors along Ayahanda Street are contaminated with coliform bacteria and Escherichia coli. Most Probable Number values exceed the microbiological safety standard of zero per 100 milliliters, indicating these ready-to-consume beverages are unfit for human consumption. The presence of fecal contamination represents a public health hazard that increases the risk of waterborne gastrointestinal disease among the local population. Immediate, coordinated action by municipal health authorities is required to enforce food sanitation and personal hygiene standards in the informal street food sector. Implementing targeted educational programs on safe water sourcing and establishing ongoing microbiological monitoring are essential to reduce these health risks and improve the safety of commercially sold beverages.

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