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Identification of *Escherichia coli* in Ice *Dawet* Sold in the Palu City Area

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Abstract

Escherichia coli is a Gram-negative bacterium from the family *Enterobacteriaceae* found in the human body and is used as an indicator of hygiene in water, food, or beverages. The purpose of this study was to identify the presence of *E. coli* in ice *dawet* sold in the Palu City area. This study used a descriptive-exploratory design with the Most Probable Number (MPN) method, Total Plate Count (TPC), and biochemical testing using Triple Sugar Iron Agar (TSIA) medium. Samples were collected from five locations in the Palu City area using simple random sampling. Based on the results, the Most Probable Number (MPN) test produced an average value of 18.700 MPN/mL of sample, while the Total Plate Count (TPC) test showed an average value of 26.800 CFU/mL of sample, indicating that the samples did not meet the standards set by the Indonesian National Standard (SNI) under the Ministry of Health, which states that the maximum allowable microbial contamination in beverages is 0/100 mL. Confirmatory tests on Eosin Methylene Blue Agar showed colony growth with a metallic green sheen and reddish coloration, characteristic of Gram-negative bacilli. The results of the Triple Sugar Iron Agar biochemical test were positive, indicating biochemical characteristics consistent with *E. coli*. Therefore, the findings confirm the presence of *E. coli* in the analyzed samples.

Keywords: Bacteria; *Escherichia coli*; Ice *dawet*.

INTRODUCTION

Escherichia coli, commonly known as *E. coli*, is a Gram-negative bacterium belonging to the family *Enterobacteriaceae* (Rahmadani, 2023). It is naturally present in the digestive systems of humans and animals and consists of both commensal strains, which are harmless, and pathogenic strains that can cause disease (Safitri et al, 2024). *E. coli* is widely used as an indicator organism for detecting fecal contamination in food and beverages. Its presence reflects poor sanitation and may lead to health problems such as diarrhea, fever, and vomiting. Transmission occurs through contamination pathways involving infected food handlers and intermediary agents such as hands, flies, soil, and water that come into contact with food or utensils (Dewi et al, 2024). As an important sanitation indicator, *E. coli* plays a key role in food safety monitoring, especially with advances in pathogen detection methods relevant to public health concerns (Zikra et al, 2018).

Food sanitation and hygiene are closely related aspects in maintaining food quality. Hygiene focuses on cleanliness practices in handling food and beverages to prevent health problems in consumers. Unsafe food may lead to infections or intoxication, often caused by contamination with *E. coli*, a bacterium naturally found in the digestive tract of humans and animals (Hutasoit, 2020). Beverage quality is strongly influenced by the cleanliness of food handlers, as poor personal hygiene can facilitate the transfer of microorganisms. In addition to human factors, water serves as a major contamination pathway, as it is used in various processes, allowing contaminated water to spread microorganisms to food and beverages.

Ice *dawet* is a traditional Indonesian dessert beverage made from coconut milk, palm sugar, and other ingredients. It is widely sold as a refreshing drink in various settings, including cafés, restaurants, and street vendors (Lorensia et al, 2024). Commonly distributed by mobile vendors, ice *dawet* is easily accessible to consumers. However, it is susceptible to contamination by pathogenic bacteria, particularly through water used in coconut milk processing and ice production. Observations in the Palu City area indicate that ice *dawet* is often sold using carts in roadside environments, increasing the risk of contamination. Additionally, the lack of proper packaging and preparation practices makes quality control difficult. Therefore, further testing is necessary to determine whether its microbial content meets safe consumption standards.

The requirement for *Escherichia coli* bacterial content in ice *dawet* beverages is, according to the Indonesian National Standard (SNI) 3553:2015, the microbial contamination requirement in ice *dawet* products is 1×10^5 colonies/mL (Safrida, 2022). According to the Indonesian National Standard (SNI), the maximum allowable microbial contamination in beverages is 0/100 mL. This provision regarding the maximum limit of microbial contamination is used as a parameter or reference in assessing the results of laboratory examinations (Ratna et al, 2019). To increase awareness and understanding among the people of Palu City regarding the risks and prevention of *E. coli* bacterial contamination in ice *dawet* beverages, this study is needed with the aim of identifying *E. coli* bacteria in ice *dawet* sold in the Palu City area.

MATERIALS AND METHODS

Equipment and Materials

The equipment used in this study included beakers, Erlenmeyer flasks, test tubes, Durham tubes, test tube racks, Petri dishes, a Bunsen burner, spatula, inoculating loop, micropipettes, autoclave, incubator, analytical balance, hot plate, glass slides, microscope, and a digital camera. The materials used in this study consisted of ice *dawet* samples, Nutrient Agar (NA), Lactose Broth (LB), Eosin Methylene Blue Agar (EMBA), and Triple Sugar Iron Agar (TSIA). Additional materials included distilled water, technical ethanol (spiritus), crystal violet solution, eosin stain, iodine, Lugol's solution, 95% alcohol, and safranin.

Methods

This study employed a descriptive exploratory design. Descriptive research aims to systematically, factually, and accurately describe a phenomenon or event. Meanwhile, exploratory research focuses on gaining deeper understanding and identifying new ideas related to a specific phenomenon. The objective is to describe social phenomena and explain the processes underlying them, with the ultimate goal of formulating more detailed research problems or developing hypotheses rather than testing them (Bambang, 2018).

Sample collection was carried out using the simple random sampling method. Simple random sampling is a technique in which each ice *dawet* sample in the population has an equal probability of being selected. This method is considered a basic sampling technique and is often used as a foundation for developing more complex sampling approaches. It can be easily applied when all members of the population are clearly identified (Syaputra, 2022).

Simple random sampling involves selecting samples based on a population list (sampling frame) to determine the sampling interval. Through this method, every member of the population has an equal chance of being included in the study. The number of samples is generally determined based on the selected level of significance (Nurdin et al, 2018). This technique was applied in the Palu City area by randomly collecting ice *dawet* beverage samples. A total of five samples sold in the Palu City area were used in this study.

Procedures

Sterilization of Equipment and Materials

To ensure that bacteria grow without contamination from other microorganisms, the cleanliness of equipment and media must be maintained. Therefore, sterilization of equipment, media, and working techniques is required. Glassware was wrapped in paper and sterilized in an oven at 160–170°C for 2 hours. Meanwhile, microbial growth media were sterilized using an autoclave at 121°C under 2 atm pressure for 15 minutes.

Preparation of Culture Media

The growth media were weighed according to the required amount based on the instructions provided on the label, then transferred into an Erlenmeyer flask containing distilled water and shaken until completely dissolved. The mouth of the flask was covered with aluminum foil and sterilized using an autoclave at 121°C for 20 minutes. After sterilization, the flask was removed and allowed to cool. The medium was then poured aseptically into Petri dishes, allowed to solidify, and subsequently stored in an incubator.

Sample Dilution

Five test tubes containing 9 mL of distilled water were prepared. One milliliter of ice *dawet* sample was added into the first tube and considered as a 10^{-1} dilution. The first tube was homogenized, then 1 mL of the suspension was transferred into the second test tube to obtain a 10^{-2} dilution, and the process was continued up to a 10^{-3} dilution. The same procedure was applied to all ice *dawet* samples.

Inoculation on Culture Media

Samples that had undergone serial dilution were incubated at 37°C for 24 hours. After incubation, 1 mL of the sample was taken using a pipette and transferred into Lactose Broth medium. The sample was homogenized in the medium and incubated again at 37°C for 24 hours. After incubation in Lactose Broth, lactose fermentation activity by coliform bacteria was indicated by gas formation and turbidity in the medium. Subsequently, the samples were cultured on Nutrient Agar using the pour plate method. The sample was transferred using a micropipette into a sterile Petri dish, followed by the addition of Nutrient Agar (NA) until the sample was covered with a layer of approximately 1 cm thickness. After solidification, the plates were incubated at 37°C for 24 hours. To identify *E. coli*, subculturing was carried out using a selective medium, Eosin

Methylene Blue Agar (EMBA). A loopful of bacterial culture grown on Nutrient Agar was aseptically streaked onto the surface of EMBA plates and incubated at 37°C for 24–48 hours. The bacterial colonies were then observed; colonies exhibiting a dark blue or metallic green color indicated the presence of *E. coli.* Furthermore, the isolates were inoculated onto Triple Sugar Iron Agar by stabbing the inoculating needle vertically into the butt (bottom of the medium), followed by streaking the slant (inclined surface) in a zig-zag pattern. The medium was then incubated at 37°C for 24 hours.

Gram Staining

The Gram staining procedure began by cleaning the glass slide with alcohol to remove grease, followed by brief heating over a Bunsen flame. A loopful of bacterial suspension was then aseptically placed onto the slide and spread evenly over an area of approximately 1 cm². The smear was air-dried and heat-fixed over a spirit lamp flame. After cooling, the smear was flooded with the primary stain (Gram A) for 60 seconds, rinsed with running water, and air-dried. The next step involved adding Gram B solution for 1 minute, followed by rinsing and drying. The smear was then treated with Gram C solution for 30 seconds, rinsed with distilled water, and air-dried. Subsequently, the counterstain Gram D (safranin) was applied for 2 minutes, followed by rinsing and drying either by air or using filter paper. Once completely dry, the stained smear was observed under a microscope at high magnification. If the observation was unclear, immersion oil was added to enhance visualization of the bacterial cells.

RESULTS AND DISCUSSION

Presumptive Test Results

The analysis of ice *dawet* beverage samples obtained from the Palu City area indicated the presence of coliform bacteria. This was evidenced by the formation of gas bubbles and turbidity in the medium. The appearance of gas bubbles in the Durham tubes along with the turbidity of the medium indicated lactose fermentation activity by coliform bacteria. Bacterial activity in Lactose Broth (LB) medium is shown in (Figure 1).

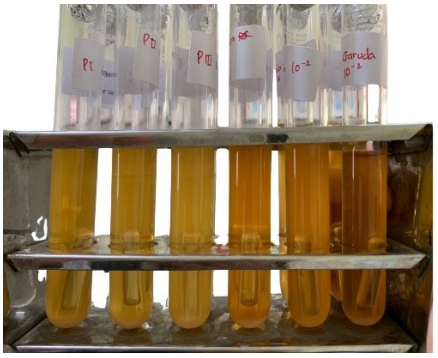


Figure 1. Color changes in Lactose Broth medium after incubation for 2 × 24 hours (48 hours).

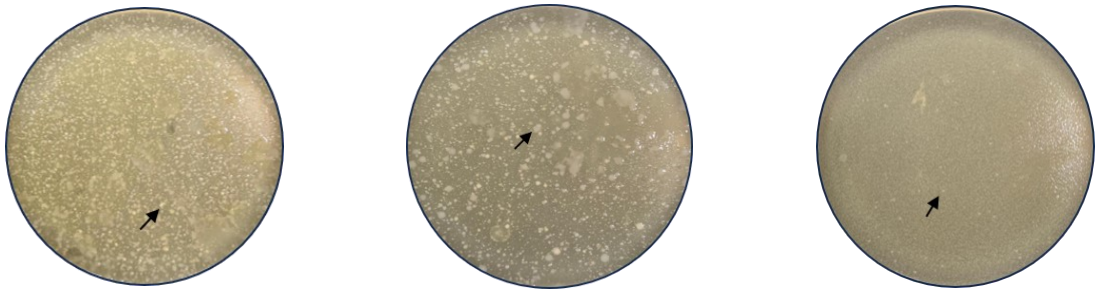
The number of bacteria was calculated using the Most Probable Number (MPN) method. The MPN method is a statistical approach used to estimate the number of bacteria in a sample. The MPN results showed that ice *dawet* sample C had the lowest MPN value of 35 × 10^2 MPN/mL, whereas sample E had an MPN value of 460 × 10^2 MPN/mL, indicating a higher level of bacterial contamination. The obtained MPN values are presented in (Table 1).

Table 1. Most Probable Number (MPN) Values of Coliform Bacteria in Lactose Broth

Sample (ice *dawet*)	Dilution			MPN
	10^{-1}	10^{-2}	10^{-2}	(MPN/mL of sample)
ice *dawet* A	3	2	2	210 x 10^2
ice *dawet* B	2	1	1	20 x 10^2
ice *dawet* C	2	2	2	35 x 10^2
ice *dawet* D	3	2	2	210 x 10^2
ice *dawet* E	3	3	1	460 x 10^2

Total Plate Count method results

Positive samples from the presumptive test in Lactose Broth were subsequently cultured on Nutrient Agar to determine the number of bacterial colonies formed. The bacterial colonies growing on Nutrient Agar are shown in (Figure 2).



(a) (b) (c)

Figure 2. Bacterial colonies on Nutrient Agar (NA). (a) Dilution 10^{-1} (b) Dilution 10^{-2} (c) Dilution 10^{-3}

Based on the Total Plate Count (TPC) method, the number of bacterial colonies growing on Nutrient Agar at dilution levels of 10^{-1} to 10^{-3} is presented in (Table 2).

Table 2. Total Plate Count Values of Bacterial Colonies.

Sample (ice *dawet*)	Dilution			TPC (CFU/mL of sample)
	10^{-1}	10^{-2}	10^{-3}	
ice *dawet* A	518	443	493	443 x 10^2
ice *dawet* B	458	328	63	328 x 10^2
ice *dawet* C	671	181	75	181 x 10^2
ice *dawet* D	156	155	40	155 x 10^2
ice *dawet* E	259	233	236	233 x 10^2

From the data above, ice *dawet* sample D showed the lowest TPC value, which was 155 × 10^2 CFU/mL. In contrast, sample A had a TPC value of 443 × 10^2 CFU/mL, indicating a higher level of bacterial contamination.

Confirmatory Test on Selective Medium

The results of bacterial cultivation on the selective medium, Eosin Methylene Blue Agar (EMB), showed the presence of bacterial colony growth. The use of this medium allows for the detection and identification of *coliform* bacteria. The bacterial colonies grown on Eosin Methylene Blue Agar are presented in (Figure 3).

Figure 3. *Escherichia coli* colonies on selective medium.

The results of this study on the characteristics of *E. coli* colonies in ice *dawet* samples (A, B, C, D, and E) sold in the Palu City area indicated that the colonies were circular in shape, dark red in color with metallic green edges. The colonies exhibited a moist and mucoid texture, with irregular margins and a convex surface.

Gram Staining Results

Gram staining was performed to observe the morphology of bacterial cells. This technique was used to differentiate and classify bacteria into Gram-positive or Gram-negative groups based on differences in their cell wall structure. Observations were conducted under a microscope at a magnification of 100×10. The results of bacterial cell morphology observed through Gram staining are shown in (Figure 4).

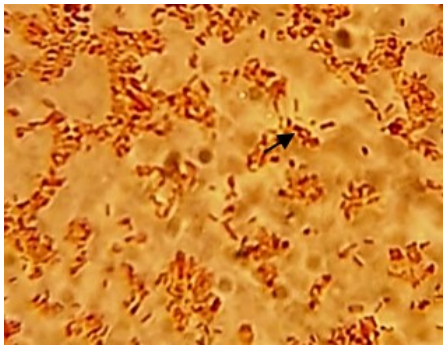


Figure 4. Gram staining results of Escherichia coli cells on selective medium

Biochemical Test Results on TSIA Medium

Bacterial colonies grown on selective medium were then taken using one loopful and subcultured onto Triple Sugar Iron Agar (TSIA) medium to determine the presence or absence of biochemical reactions in the bacteria. The results of the biochemical analysis are presented in (Table 3).

Table 3. Biochemical Test Results on TSIA Medium.

Bacterial Sample	*Triple Sugar Iron Agar* Medium			H_2S
	Butt	Slant	Gas	
Escherichia coli	A	A	+	+

Description:
A = Acid (yellow medium)
B = Base (red medium)
H_2S = Black coloration at the bottom of the tube
Gas = Presence of gas bubbles
\+ = Positive (producing gas and H_2S)
− = Negative (not producing gas and H_2S)

Discussion

The determination of coliform bacterial contamination in ice *dawet* beverages using the Most Probable Number method with Lactose Broth medium showed that, at dilution levels of 10^{-1} to 10^{-3}, samples collected from five different locations in the Palu City area produced varying results. The Most Probable Number is a method used to estimate bacterial growth in prepared liquid media (Zahra, 2019). This method consists of three main stages, namely the presumptive test to detect the general presence of coliform bacteria, the confirmatory test to verify specific coliform presence, and the completed test (Misrofah & Purwantisari, 2021). The MPN values obtained were 21.000 MPN/mL for ice *dawet* sample A, 2.000 MPN/mL for sample B, 3.500 MPN/mL for sample C, 21.000 MPN/mL for sample D, and 46.000 MPN/mL for sample E, with an overall average of 18.700 MPN/mL. In the MPN method, Lactose Broth (LB) serves as the presumptive test medium to determine the presence of coliform bacteria based on acid and gas formation resulting from lactose fermentation. Positive results in this test are indicated by gas formation in the Durham tubes and acid production marked by changes in

the medium (Fatimah & Andriani, 2024). The MPN results indicate significant contamination of coliform bacteria in ice *dawet* beverages. Therefore, all five ice *dawet* samples tested had coliform MPN values exceeding the established maximum threshold and can be considered as not meeting the applicable food safety standards. According to the Indonesian National Standard (SNI), the maximum allowable microbial contamination in beverages is 0/100 mL. This standard serves as a reference for evaluating laboratory examination results (Ratna et al, 2019). Furthermore, based on the Regulation of the Minister of Health of the Republic of Indonesia No. 2 of 2023, the permissible total coliform bacteria in food and beverages is 0/100 mL of sample (Musdalifah et al, 2024).

The Total Plate Count (TPC) method is a technique used to determine the number of microorganisms present in a sample or preparation (Irfan & Jufri, 2021). The TPC values obtained were 44.300 CFU/mL for ice *dawet* sample A, 32.800 CFU/mL for sample B, 18.100 CFU/mL for sample C, 15.500 CFU/mL for sample D, and 23.300 CFU/mL for sample E, with an overall average of 26.800 CFU/mL per sample. According to the Indonesian National Standard (SNI) 3553:2015, the acceptable microbial contamination limit in ice *dawet* products is 1×10^5 colonies/mL (Safrida, 2022). Thus, based on the obtained microbial contamination levels in all tested ice *dawet* samples, the TPC values were still below the maximum allowable limit. Based on the TPC values obtained from the five ice *dawet* samples and supported by previous studies, the microbiological quality of ice *dawet* beverages is strongly influenced by hygiene and sanitation practices during processing and serving. Proper implementation of hygiene measures can ensure that ice *dawet* is safe for consumption.

Eosin Methylene Blue Agar (EMBA) is a differential medium used to identify *E. coli*. This medium supports the growth of bacteria belonging to the Enterobacteriaceae group, including *E. coli*. On EMBA, *E. coli* typically forms colonies characterized by a circular shape, approximately 2–3 mm in diameter, with a metallic green color and a bluish-green center (Fatiqin et al, 2019). EMBA contains lactose and sucrose and functions as a highly selective medium for isolating or identifying Gram-negative bacteria. One of the pathogenic bacteria from the *coliform* group, *E. coli*, produces colonies with a metallic green appearance on EMBA. This occurs because *E. coli* is capable of fermenting lactose in the medium, resulting in acid production (Mayanti et al, 2023). Based on the observations in this study, the bacterial colonies exhibited characteristic morphology, including a circular shape, smooth and convex surface, entire margins, and a dark red color with metallic green edges. These findings are consistent with previous studies reporting that *E. coli* colonies show a metallic green sheen, rod-shaped morphology, Gram-negative characteristics, and entire margins with convex elevation (Sari et al, 2019).

The Gram staining results showed that *E. coli* belongs to the Gram-negative group, characterized by short rod-shaped cells resembling chains and appearing reddish in color. These findings are consistent with those reported by Marbun et al, (2020), who described *E. coli* as bacilli with a reddish appearance. This characteristic is associated with the structure of the Gram-negative bacterial cell wall, which contains a thin peptidoglycan layer and a lipid-rich outer membrane that cannot retain the primary stain (crystal violet). During the staining process, the addition of iodine does not effectively fix the dye, and subsequent decolorization with alcohol disrupts the lipid layer, causing the primary stain to be washed out. As a result, the cells absorb the counterstain and appear red or pink. Similar observations were also reported by Arnawa (2023), who described *E. coli* as short rod-shaped bacteria with a reddish coloration.

The main objective of the TSIA test is to differentiate genera of Gram-negative bacteria within the *Enterobacteriaceae* group based on their ability to ferment glucose. This test also serves to distinguish *Enterobacteriaceae* from other Gram-negative bacilli found in the intestinal tract (Widyastuti et al, 2022). Changes indicating the growth of *E. coli* are marked by a yellow coloration throughout the medium, both in the butt (deep portion) and the slant (surface), due to the ability of *E. coli* to ferment glucose, lactose, and sucrose. In addition, sodium thiosulfate present in the medium acts as a substrate for detecting hydrogen sulfide (H_2S) production, where the formation of a black precipitate serves as an indicator distinguishing it from other bacteria (Supriadi et al, 2023). Observations in this study showed that the bacteria were capable of fermenting glucose, lactose, and sucrose, indicated by a color change from red to yellow, accompanied by gas formation and the appearance of black precipitate indicating H_2S production. Positive TSIA results for *E. coli* are characterized by a yellow color in both the slant and butt, along with gas production that may cause the medium to crack or lift (Khoiriyah & Busman, 2023).

The tested ice *dawet* beverages showed a high level of coliform contamination and were considered unsafe for consumption. Therefore, maintaining hygiene and sanitation in food and beverages is essential, starting from the cleanliness of equipment and raw materials, through processing and serving, as well as ensuring a clean selling environment to prevent bacterial contamination. Continuous efforts are required to maintain the quality and safety of ice *dawet* by implementing stricter sanitation standards. In general, ice *dawet* is sold without specialized packaging and is often prepared directly at the point of sale, making quality control difficult. Microbial contamination may occur at various stages, particularly during processing and from raw materials. This is often associated with inadequate hygienic practices, including improper handling and poor hand hygiene among workers. In addition, environmental factors such as air, water, dust, waste, and decomposing

organic matter may serve as sources of contamination. As a result, ice *dawet* can become contaminated with microorganisms and pose health risks to consumers (Putri et al, 2024).

Ice dawet is a widely consumed beverage made from starch-based components, coconut milk, and palm sugar, typically served with ice. Surveys conducted in markets and vendor locations indicate that some sellers have not fully implemented proper hygiene and sanitation practices, including personal hygiene. This condition increases the risk of contamination by pathogenic bacteria such as *E. coli*. Contamination may originate from water used in coconut milk preparation or ice production and can occur throughout processing and distribution stages. Additionally, inadequate handwashing practices among workers contribute to microbial contamination in ice *dawet* (Putri et al, 2024). To reduce microbial growth in processed foods, all food handling facilities must implement proper sanitation and hygiene practices. Food and beverage sanitation is a preventive measure that emphasizes protecting food from potential hazards that may compromise health. These efforts should be applied at all stages, including pre-production, processing, storage, distribution, and final consumption by the public (Sitaba et al, 2022).

CONCLUSIONS

Based on the results of this study, it can be concluded that the dawet ice sold in the Palu City area is suspected to be contaminated with *E. coli*. The ice *dawet* sold in this area does not meet microbiological health standards because the maximum allowable coliform contamination in beverages is 0/100 mL, whereas the MPN value obtained reached 18.700 MPN/mL of sample, exceeding the maximum limit established by the Indonesian National Standard (SNI) under the Ministry of Health. Therefore, improvements in sanitation and hygiene are required, along with routine bacteriological examinations conducted by the relevant authorities.

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Competing Interests: The authors declare that there are no competing interests.

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