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Identification of *Escherichia coli* in *Gado-Gado* Sold Around Tadulako University

Nur Amal, I Nengah Kundera*, Syech Zainal, Yulia Windarsih, Rafiqa, Abdul Ashari
Department of Biology Education, Faculty of Teacher Training and Education, Tadulako University.
Jl. Soekarno Hatta No KM 9, 94148, Central Sulawesi, Tel./Fax. (0451)422611, Indonesia.

Corresponding author*
nengahkundera@gmail.com

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Abstract

Escherichia coli is a pathogenic bacterium that has the potential to contaminate food. Certain strains of *E. coli* exhibit pathogenic properties and may cause infections in hosts. The presence of pathogenic bacteria in food is a critical concern in food safety. This study aimed to identify the presence of *E. coli* in *gado-gado* sold in the canteens of Tadulako University. This research employed a descriptive exploratory laboratory approach. Samples were collected from five different canteens using random sampling techniques. Microbiological analyses were conducted using the Most Probable Number (MPN) method, Total Plate Count (TPC), Gram staining, cultivation on selective Eosin Methylene Blue Agar, and biochemical testing using Triple Sugar Iron Agar. The results showed that the average Most Probable Number was 4,900 MPN /mL of sample, while the Total Plate Count averaged 35,800 CFU/mL. This TPC value exceeds the standard set by the Indonesian Food and Drug Authority (BPOM) of 10^4 CFU/g, indicating that the food products do not meet the required health standards. Confirmatory tests on Eosin Methylene Blue Agar showed colony growth with a metallic green sheen and reddish coloration, characteristic of Gram-negative bacilli. Biochemical testing on Triple Sugar Iron Agar yielded positive results consistent with the biochemical characteristics of *E. coli*. Therefore, the findings confirm the presence of *E. coli* in the analyzed samples.

Keywords: Bacteria; *Escherichia coli*; Gado-Gado.

INTRODUCTION

Escherichia coli is a Gram-negative bacterium belonging to the family *Enterobacteriaceae* (Kolopita et al, 2022). It comprises diverse strains, some of which are pathogenic and capable of causing infections in humans (Elsie & Harahap, 2016). Infections caused by *E. coli* may result in various health problems, including diarrhea, meningitis, and urinary tract infections (Trisno et al, 2019). The contamination of food by *E. coli* is a major concern in food safety, as its presence in food products poses significant health risks and renders them unsafe for consumption. Consequently, regulations have been established to control permissible levels of *E. coli* in food and beverages. According to the Regulation of the Minister of Health of the Republic of Indonesia No. 1096/MENKES/PER/VI/2011, the acceptable level of *E. coli* is 0 CFU/g, indicating that it must be completely absent from food products (Permatasari et al, 2021).

E. coli can spread easily through contaminated water, subsequently infecting or contaminating food that comes into contact with it. This bacterium is commonly found in various food products such as chicken, eggs, and milk, particularly when they are inadequately cooked or not properly heat-treated. *Gado-gado* is a traditional Indonesian dish composed mainly of boiled vegetables, complemented with ingredients such as rice cake (lontong), tempeh, tofu, eggs, cucumber, and crackers, and served with peanut sauce as its distinctive element. This dish is widely known for being practical, nutritious, affordable, and well accepted across different social groups as a healthy and economical food option. However, gado-gado is categorized as a food product in which many components do not undergo a final heating process before consumption. This condition increases its susceptibility to contamination by pathogenic microorganisms, including E. coli, especially when food handling and sanitation practices are inadequate (Nurhayati et al, 2024). Its high popularity among students and academic communities at Tadulako University highlights the importance of food safety, as potential contamination may pose health risks to regular consumers.

Tadulako University is a higher education institution located in Palu City, Central Sulawesi, Indonesia. It is recognized as one of the largest universities in the region and plays an important role in developing qualified human resources and supporting regional and national development. In addition to academic activities, the university provides various supporting facilities for

students, faculty, and staff, including campus canteens that supply food and beverages for the academic community. As a higher education institution, Tadulako University has a responsibility to ensure that food provided in its canteens is safe, hygienic, and free from contamination. Therefore, the implementation of healthy canteen standards is essential to maintain food quality and safety. However, in practice, the university canteens have not yet fully met the expected criteria of a healthy canteen, indicating the need for improved sanitation and food safety management.

One of the key requirements for achieving healthy canteen standards is providing food and beverages that are hygienic, nutritious, safe, and compliant with established health and food safety regulations. Food safety is essential as it directly affects consumer health; therefore, food must be free from harmful chemical substances, such as unauthorized additives and excessive preservatives. In addition, food should be free from microbiological contamination, particularly pathogenic bacteria that can cause foodborne diseases (Rachmawaty et al, 2024). One of the most commonly reported pathogens in food safety studies is *E. coli*, due to its ability to contaminate food and cause illness. To date, no specific data are available regarding *E. coli* contamination in *gado-gado* sold in Tadulako University canteens. Therefore, this study aimed to identify its presence.

MATERIALS AND METHODS

This study employed a descriptive exploratory laboratory approach. *Gado-gado* samples were obtained by purchasing them from five different canteens at Tadulako University. The sampling technique used in this study was random sampling to ensure representative sample selection.

The equipment used in this study included beakers, Erlenmeyer flasks, test tubes, Durham tubes, test tube racks, a Bunsen burner, spatula, inoculating loop, Petri dishes, incubator, autoclave, micropipettes, analytical balance, hot plate, microscope, and glass slides. The materials used consisted of gado-gado samples, Lactose Broth, Nutrient Agar, Eosin Methylene Blue Agar (EMB Agar), Triple Sugar Iron Agar (TSI Agar), distilled water (aquades), and Gram staining reagents, including crystal violet, iodine, alcohol, and safranin.

Procedures

Sterilization of Equipment and Media

The research procedures began with a preparation stage involving the sterilization of all laboratory equipment used in the study. The equipment included test tubes, Durham tubes, graduated cylinders, Erlenmeyer flasks, Pasteur pipettes, glass slides, inoculating loops, and Petri dishes. All equipment was first wrapped in paper to maintain cleanliness and prevent external contamination. Subsequently, the wrapped equipment was sterilized using an autoclave for approximately 2 hours. Autoclave sterilization was performed to ensure that all equipment was free from microorganisms, including bacteria and fungi, thereby preventing interference with the experimental results.

In addition to the equipment, all culture media used in this study were also sterilized prior to use. The prepared media included Lactose Broth, Nutrient Agar, Eosin Methylene Blue Agar, and Triple Sugar Iron Agar. Media sterilization was carried out by autoclaving at 121°C for 15 minutes. The high temperature and pressure generated under these conditions are effective in eliminating contaminating microorganisms, ensuring that the media were sterile and suitable for subsequent inoculation and microbiological analysis.

Preparation of Culture Media

The culture media were prepared by weighing the required amount of medium according to the number of samples and the type of analysis to be performed. The weighed media were then transferred into Erlenmeyer flasks containing distilled water as a solvent. The mixture was homogenized by stirring until a uniform solution was obtained, ensuring the absence of clumps or precipitates that could affect medium quality. The Erlenmeyer flasks were subsequently heated on a hot plate with gentle agitation to facilitate complete dissolution and homogenization of the media. The flask openings were then plugged with cotton and covered with aluminum foil to prevent contamination while allowing steam exchange during sterilization. Sterilization of the prepared media was carried out using an autoclave at 121°C for 15 minutes. The high temperature and pressure under these conditions effectively eliminated any contaminating microorganisms, ensuring that the media were sterile and suitable for inoculation and microbiological analysis. After sterilization, the flasks were removed from the autoclave and allowed to cool to a safe handling temperature before further use.

Isolation and Serial Dilution

The *gado-gado* samples were prepared by weighing 1 g of each sample, followed by homogenization to facilitate uniform suspension. Subsequently, 1 mL of distilled water was added to the homogenized sample to obtain an initial suspension. Serial dilution were then performed using three test tubes, each containing 9 mL of distilled water. For each sample, 1 mL of the initial suspension was transferred into the first tube to obtain a 10^{-1} dilution. After thorough mixing, 1 mL from the first tube was transferred into the second tube to obtain a 10^{-2} dilution, followed by transferring 1 mL into the third tube to achieve a 10^{-3} dilution. This serial dilution procedure was repeated for all samples collected from

different locations. The diluted samples were subsequently used for inoculation.

Inoculation and Bacterial Cultivation

The diluted samples were inoculated into Lactose Broth medium containing Durham tubes by transferring 1 ml of each dilution using a micropipette. The inoculated media were incubated at 37°C for 24 hours. Positive results indicating bacterial presence were identified by gas formation in the Durham tubes and/or turbidity in the Lactose Broth. Samples showing positive results were further cultured on Nutrient Agar using the pour plate method. The sample suspension was transferred into sterile Petri dishes, followed by the addition of molten Nutrient Agar. After solidification, the plates were incubated at 37°C for 24 hours. Identification of *Escherichia coli* was carried out by subculturing bacterial colonies onto Eosin Methylene Blue (EMB) Agar. A loopful of culture from Nutrient Agar was aseptically streaked onto the surface of solidified EMB Agar plates and incubated at 37°C for 24–48 hours. Following incubation on EMB Agar and colony formation, further identification was conducted using Triple Sugar Iron (TSI) Agar to evaluate the ability of the bacteria to produce acid and gas. A loopful of bacterial culture from EMB Agar was inoculated into TSI Agar by stabbing approximately three-quarters of the depth of the medium and streaking the slant surface. The inoculated media were then incubated at 37°C for 24 hours..

Gram Staining

Gram staining was performed using bacterial colonies grown on selective Eosin Methylene Blue (EMB) Agar. A loopful of bacterial colony was aseptically taken and evenly smeared onto a clean glass slide to form a thin film suitable for microscopic observation. The smear was then heat-fixed by gentle heating to ensure that the bacteria adhered firmly to the slide and were not removed during the staining process. The staining procedure began with the application of crystal violet as the primary stain. The smear was flooded with crystal violet and allowed to stand for 1 minute, followed by rinsing with running water to remove excess stain. Subsequently, iodine solution was added as a mordant to form a crystal violet–iodine complex within the bacterial cell wall. After 1 minute, the slide was rinsed again with running water. Decolorization was then performed by applying alcohol for approximately 30 seconds. This step dissolves lipids in the cell wall of Gram-negative bacteria, allowing the crystal violet–iodine complex to be washed out of the cells. The slide was immediately rinsed with water to stop the decolorization process. The final step involved counterstaining with safranin, which was applied for 2 minutes and then rinsed with water. The slide was gently dried using tissue paper without touching the smear surface. A drop of immersion oil was added, and the stained preparation was observed under a microscope using 100× magnification (oil immersion) (Dewi et al, 2022).

Data analysis

Data analysis in this study was conducted using a descriptive exploratory approach. A quantitative approach was applied through the Most Probable Number (MPN) method using a 3–3–3 tube series system in Lactose Broth to detect the presence of coliform bacteria. Observations of gas formation and turbidity were recorded and interpreted using standard MPN tables to estimate microbial density (CFU/mL sample). In addition, the Total Plate Count (TPC) method on Nutrient Agar was employed to determine the total viable bacterial count. Colony counts obtained from the plates were used to calculate the number of colony-forming units per milliliter (CFU/mL). Identification of *E. coli* was carried out based on colony characteristics observed on Eosin Methylene Blue (EMB) Agar, as well as morphological analysis through Gram staining. Biochemical testing using Triple Sugar Iron (TSI) Agar was performed to evaluate the metabolic capability of the bacteria in fermenting sugars and producing gas and hydrogen sulfide (H_2S).

RESULTS AND DISCUSSION

Presumptive Test (Most Probable Number)

The initial stage of the Most Probable Number (MPN) analysis began with the presumptive test, a procedure aimed at detecting the presence of coliform bacteria based on their ability to ferment lactose in liquid Lactose Broth medium. This step is essential as it provides an initial indication of whether the tested samples contain *coliform* bacteria, including *Escherichia coli*, which are commonly used as indicators of microbiological contamination in food (Dwitami et al, 2024). In this study, a 3–3–3 tube series system was employed, meaning that each sample was tested using three tubes at each dilution level. The inoculated tubes were incubated at 37 °C for 24 hours, which is the optimal temperature for *coliform* bacterial growth. Gado-gado samples obtained from five different canteens within Tadulako University were analyzed using this method. The results of the presumptive test indicated that all samples were positive for bacterial contamination, as evidenced by observable changes in the Lactose Broth medium. Positive indications of coliform presence were demonstrated by the formation of gas bubbles in the Durham tubes and/or turbidity in the broth medium (Figure 1). All five samples showed positive results at each dilution level (Table 1). These positive results were subsequently used to determine microbial density by referring to standard Most Probable Number (MPN) tables.

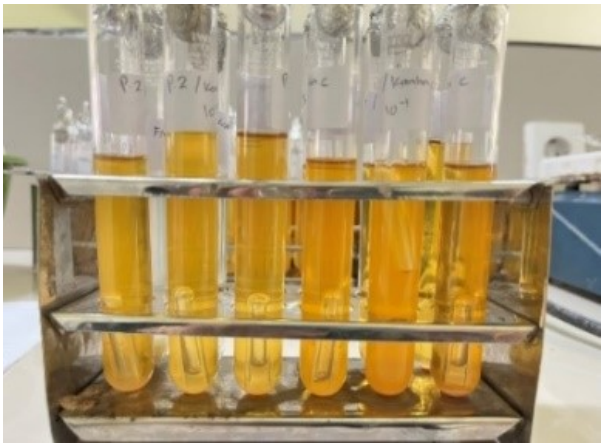


Figure 1. Positive presumptive test in Lactose Broth after incubation, indicated by gas formation and turbidity as evidence of coliform bacterial activity

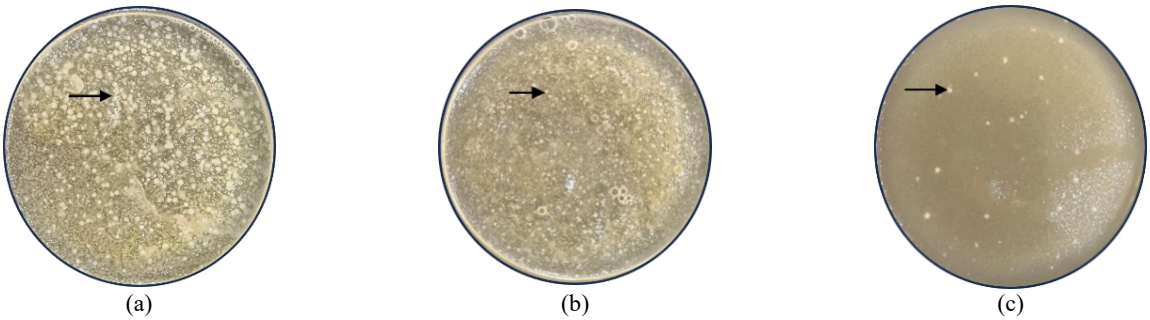
Table 1. Most Probable Number (MPN) Values of Coliform Bacteria in Lactose Broth.

Sample (Gado-Gado)	Dilution Tubes			MPN (MPN/mL sample)
	10^{-1}	10^{-2}	10^{-3}	
Canteen A	2	1	**1**	20 x 10^2
Canteen B	2	1	1	20 x 10^2
Canteen C	2	2	2	35 x 10^2
Canteen D	2	1	1	20 x 10^2
Canteen E	3	2	1	150 x 10^2

The results of the Most Probable Number calculation on gado-gado from five canteens at Tadulako University showed variation. The Most Probable Number value is used as an indicator of hygiene level and food safety based on the number of bacteria detected. Canteen E has the highest value, namely 150 × 10^2 MPN/mL of sample, indicating the highest contamination. In contrast, Canteens A, B, and D have the lowest value of 20 × 10^2 MPN /mL of sample. Canteen C is in the middle with 35 × 10^2 MPN /mL of sample.

Total Plate Count Method Results

Samples that showed positive results at the presumptive test stage using Lactose Broth medium were then continued to the next stage, namely the rejuvenation process into Nutrient Agar medium. This stage was carried out with the aim of regrowing the bacteria that had been detected, while also ensuring that the number of colonies formed could be counted more clearly and accurately. The growth of bacterial colonies on Nutrient Agar medium produced colonies that could be observed directly. With the presence of clear and separated colonies, the researcher could perform the calculation of the number of bacterial colonies, which was then used as the basis for assessing the level of contamination in the gado-gado samples (Figure 2).



(a) (b) (c)

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

The results of the calculation of the number of bacterial colonies that grew on Nutrient Agar medium, based on the Total Plate Count method or plate count with dilution levels of 10^{-1} to 10^{-3} (Table 2).

Table 2. Total Plate Count Values of Bacterial Colonies.

Sample (Gado-Gado)	Dilutions Tubes			Total Plate Count (CFU/mL sample)
	10^{-1}	10^{-2}	10^{-3}	
Canteen A	853	220	42	220 x 10^2
Canteen B	502	418	241	418 x 10^2
Canteen C	382	215	48	215 x 10^2
Canteen D	571	537	266	537 x 10^2
Canteen E	581	406	256	406 x 10^2

The results of the calculation of the number of bacterial colonies using the Total Plate Count method on *gado-gado* from five canteens at Tadulako University showed variation. The highest value was found in Canteen D, 537×10² CFU/mL of sample, indicating the highest contamination. In contrast, the lowest value was found in Canteen C, 215 × 10² CFU/mL of sample, indicating the lowest level of contamination compared to the other canteens.

Confirmatory Test on Selective Medium

The results of bacterial inoculation on selective Eosin Methylene Blue Agar showed the growth of colonies with a dark red color with metallic green edges and different characteristics on each medium. The colonies formed on Eosin Methylene Blue Agar medium (Figure 3).

Figure 3. Bacterial Colonies on Eosin Methylene Blue Agar

Based on the figure, bacterial colonies grown on Eosin Methylene Blue Agar medium can be observed. The figure above shows bacterial colonies on this medium. The results of the study on the characteristics of *Escherichia coli* colonies in *gado-gado* sold in canteens at Tadulako University indicate that the bacteria have characteristics in the form of circular colony shape, with red colony color with metallic green edges, having a mucoid and moist texture with irregular colony margins and having elevation or a convex surface.

Gram Staining

The Gram staining process has the purpose of determining bacterial classification based on the characteristics of their cell wall, so that it can be identified whether an isolate belongs to the Gram-positive or Gram-negative group. The staining was carried out using a light microscope equipped with an objective lens with 100× magnification, allowing detailed observation of cell morphology. Through this procedure, bacteria that have a thick peptidoglycan layer will retain the primary crystal violet stain and appear purple (Gram-positive), whereas bacteria with a thin peptidoglycan layer and the presence of an outer membrane will lose the primary stain and take up the counterstain safranin, thus appearing pink (Gram-negative) (Figure 4).

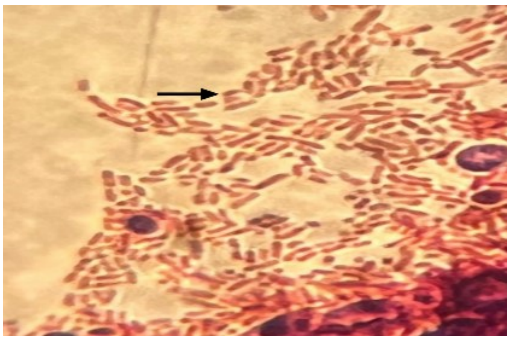


Figure 4. *Escherichia coli* Bacterial Cells in Gram Staining

Triple Sugar Iron Agar Biochemical Test

Bacterial colonies that had grown on selective medium were then taken using one loopful and inoculated onto Triple Sugar Iron Agar medium to observe the presence of biochemical reactions in the bacterial type. The results of the biochemical analysis can be seen in (Table 3).

Table 3. Results of Biochemical Reaction Analysis on Triple Sugar Iron Agar Medium.

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

Description:
A = Acid (yellow medium)
B = Base (red medium)
H_2S = Black color at the bottom of the tube
Gas = Presence of gas bubbles
\+ = Positive (producing gas and H_2S)
\- = Negative (producing gas and H_2S)

Discussion

Coliform bacterial contamination in gado-gado using the Most Probable Number method with Lactose Broth medium produced values reaching an average of 4,900

CFU/mL of sample. Lactose Broth is one of the media used to detect the presence of coliform bacteria. Indications of coliform presence are shown through acid formation that causes turbidity in the medium as well as gas accumulation in the Durham tubes (Dwitami et al, 2024). The presence of these bubbles is an indicator that lactose fermentation is occurring in the Lactose Broth medium by *coliform* bacteria (Pangesti & Indrayudha, 2025). This high MPN value indicates the presence of *coliform* contamination in gado-gado. Based on the Indonesian National Standard (SNI) No. 7388 of 2009 concerning the Maximum Limit of Microbial Contamination in Food, the maximum allowable coliform contamination in processed food products is 10^1 CFU/g (Zelpina et al, 2020). Thus, the five *gado-gado* samples have coliform MPN values above the established threshold, and therefore do not meet the applicable food safety standards. The presence of *Escherichia coli* bacteria in all *gado-gado* samples from five canteens in the environment of Tadulako University provides an overview of food safety in the campus area. The presence of this bacterium is not merely an indicator of contamination, but also reflects poor sanitation and hygiene conditions, especially in canteen facilities. This high MPN value is most likely influenced by contamination during the processing stage, considering that *gado-gado* involves ingredients that do not undergo complete heat treatment (only briefly boiled) and peanut sauce that is susceptible to bacterial exposure if not stored at the proper temperature or if the water used for washing vegetables is contaminated (Anita et al, 2022).

The Total Plate Count calculation in *gado-gado* showed an average of 35,800 CFU/mL of sample. Based on microbiological food feasibility standards in Indonesia regulated by the Indonesian Food and Drug Authority (BPOM) No. 3 of 2026 concerning the Maximum Limit of Microbial Contamination in Processed Food, which is 10^4 CFU/g (BPOM, 2026). Thus, based on the level of microbial contamination in the five *gado-gado* samples tested, the TPC values of the samples are above the established threshold, and therefore do not meet the applicable food safety standards. The Total Plate Count results being above the threshold remain a concern for consumers in the campus environment due to the potential risk of gastrointestinal infections (Wahyuli et al, 2025).

The results of bacterial cultivation on selective media showed bacterial colonies with different colors, shapes, and characteristics on each medium, indicating the presence of variation in bacterial types. Observations on Eosin Methylene Blue Agar showed that bacterial colonies had characteristic morphology in the form of circular shape, convex and smooth surface, with entire margins and dark red color with metallic green edges. This is in accordance with research conducted by (Bria et al, 2022), where *E. coli* isolates showed characteristics such as circular colonies, convex surfaces, and smooth edges. Through confirmatory testing on Eosin Methylene Blue Agar, *E. coli* bacteria show a metallic green sheen at the colony edges (Ardani et al, 2025).

In Gram staining, *E. coli* is shown to belong to the Gram-negative bacterial group with a morphology of short rod-shaped cells resembling chains, appearing pink to reddish in color. This is in line with research conducted by (Haskito et al, 2024), where the results of Gram staining showed that *E. coli* bacteria have a morphology in the form of short rods, red in color, which is a characteristic of the Gram-negative group. The cell wall of Gram-negative bacteria is composed of a lipid layer that is easily damaged when washed with alcohol. As a result, in the Gram staining process, these bacteria are unable to retain crystal violet and will appear pink after being stained with safranin. This is also consistent with research by (Alim et al, 2025), which states that Gram-negative bacteria lose the ability to retain crystal violet after the decolorization stage. As a result, the cells absorb safranin and appear red or pink when observed under a microscope. This is caused by the structure of the cell wall, which only has a thin peptidoglycan layer and an outer membrane in the form of lipopolysaccharide that is easily damaged by alcohol treatment (Dewi et al, 2022).

Triple Sugar Iron Agar medium is used to observe the ability of bacteria to ferment sugars. The growth of bacteria on Triple Sugar Iron Agar medium shows a color change in the butt and slant portions to yellow. This indicates that the bacteria growing on Triple Sugar Iron Agar medium are able to ferment sugars. The color change of the Triple Sugar Iron Agar medium from red to yellow indicates the presence of *E. coli*, because this bacterium is able to ferment lactose, sucrose, and glucose contained in the Triple Sugar Iron Agar medium (Haskito et al, 2024). The results of observations on this medium show that these bacteria are able to ferment glucose, lactose, and sucrose, as indicated by a color change from red to yellow, as well as the formation of black precipitate indicating H_2S production, and gas formation. Positive Triple Sugar Iron Agar results for *E. coli* are indicated by a yellow color change in both the slant and butt portions of the medium, as well as gas formation in the butt causing the medium surface to be lifted (Khoiriyah et al, 2022).

Contamination of *E. coli* bacteria in *gado-gado* in canteens at Tadulako University occurs due to low sanitation and hygiene quality, especially in canteen facilities. The contributing factors include the presence of open trash bins without separation of organic and inorganic waste, inadequate handwashing facilities such as the absence of sinks, soap, and running water, as well as poor personal hygiene observed during food serving, where vendors' hands come into direct contact with food, thereby increasing the risk of microbial contamination (Rohmah et al, 2018). This condition increases the likelihood of contamination. In addition, food storage

does not meet standards, for example food is left uncovered or not maintained at proper temperatures, thereby promoting bacterial growth. Temperature is also important in food storage; the storage temperature for food ingredients such as vegetables and plant-based side dishes (tofu, tempeh) should be maintained at 8–9°C to prevent the growth of pathogenic microorganisms (Jannah & Rahma, 2025). Meanwhile, canteens with good sanitation are those that have clean and enclosed kitchen facilities, food storage areas protected from pests, handwashing stations with running water, dishwashing facilities, and waste disposal systems that meet standards (Fadillah et al, 2025).

To prevent contamination by pathogenic microorganisms, strict hygiene practices are required. One of the main steps is handwashing, as food handlers' hands can act as a medium for transmitting foodborne diseases through cross-contamination. Washing hands with soap has been proven to be more effective in removing microorganisms than using water alone, and all types of soap can be used, including liquid soap, bar soap, and soap containing disinfectants (Widawati et al, 2024; Megaputri & Septiati, 2023). In addition, the cleanliness of cooking utensils and eating utensils is also very important. Utensils that are not properly washed can become a source of bacterial contamination that transfers to food during processing (Sa'adah & Hadi, 2026). Therefore, utensil washing must be carried out using proper methods to prevent the transfer of pathogenic microorganisms to food. Finally, food should be reheated thoroughly before consumption to prevent microbial growth (Halimatussadiyah et al, 2024).

CONCLUSIONS

Gado-gado sold in the environment of Tadulako University was positive for the presence of *E. coli* in all tested samples. This indicates that the gado-gado does not meet microbiological health standards, as shown by the Total Plate Count value with an average of 35,800 CFU/mL across all samples. This value exceeds the maximum threshold of microbial contamination established by the Indonesian Food and Drug Authority (BPOM) No. 3 of 2026, which is 10^4 CFU/g. Therefore, efforts are required to improve sanitation and hygiene, as well as the implementation of routine bacteriological examinations by authorized institutions.

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

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