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Identification of *Salmonella* spp. Contamination in *Gado-Gado* Sold Around the Tadulako University Campus

Pebi Angreni, I Nengah Kundera*, Yulia Windarsih, Mohammad Jamhari, Vita Indri Febriani, 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

Salmonella spp. are Gram-negative rod-shaped bacteria commonly found in the gastrointestinal tract of humans and animals and are recognized as one of the major causes of foodborne diseases. This study aimed to identify the presence of *Salmonella* spp. in *gado-gado* sold in the canteens of Tadulako University. This research employed a descriptive exploratory laboratory design. Samples were collected from five different canteens using a random sampling technique. Microbiological analyses were conducted through presumptive and confirmatory tests using the Most Probable Number (MPN) method, Total Plate Count (TPC), Gram staining, cultivation on selective *Salmonella–Shigella Agar* (SSA), and biochemical testing using Triple Sugar Iron Agar (TSIA). The MPN results showed an average value of 4,900 MPN/mL of sample, while the TPC reached 35,800/ml sample. These values indicate that the food product does not meet the microbiological safety standards established by the Indonesian Ministry of Health, as the TPC exceeds the maximum permissible limit of 10^4 CFU/mL.

Keywords: Bacteria; *Salmonella* sp; Gado-gado.

INTRODUCTION

Salmonella spp. are rod-shaped, Gram-negative, non-spore-forming bacteria capable of growing within a pH range of 4.0–9.0, with an optimal pH of approximately 7.0. These bacteria are transmitted through animals or contaminated food and enter the human body via the oral route, causing infection (Solikhah et al, 2021). Contamination is commonly associated with poor sanitation, prolonged food storage, and inadequately protected utensils. Infection caused by *Salmonella* spp., known as salmonellosis, leads to symptoms such as fever, abdominal pain, diarrhea, and vomiting, while typhoid fever remains a major public health concern (Armah et al, 2021). *Gado-gado*, a traditional Indonesian ready-to-eat dish, has a high risk of contamination due to its composition. The peanut sauce, rich in proteins and lipids, supports bacterial growth, while cross-contamination may occur through raw vegetables, washing water, contaminated utensils, and poor hygiene practices (Purwati et al, 2024; Jovanus et al, 2025).

Salmonella spp. contamination frequently occurs in processed foods such as nuggets, sausages, and undercooked eggs sold in canteens, mainly due to cross-contamination during preparation, improper storage temperatures (above 4°C), and inadequate sanitation of equipment. These Gram-negative bacteria can persist under such conditions and cause intestinal infections, particularly among vulnerable groups such as school children, with symptoms including fever, diarrhea, and abdominal cramps. National food safety standards require a negative result (0 per 25 g of sample) for *Salmonella* spp. in processed foods (Mirawati, 2014). Therefore, food products must be free from bacterial contamination to ensure safety and prevent foodborne illnesses. Contamination commonly occurs through raw materials (meat, eggs, milk), unclean utensils, or improper storage, allowing rapid bacterial proliferation and leading to gastrointestinal infections within 12–72 hours.

Tadulako University is one of the largest higher education institutions in Central Sulawesi, providing facilities to support students' needs, including campus canteens. These canteens supply various food and beverages and play a crucial role in meeting the daily nutritional needs of students and staff. Therefore, canteens must comply with healthy canteen standards (Rofiqoh et al, 2025). As part of this program, canteens are required to implement strict hygiene practices, such as washing utensils with running water and soap and storing them in enclosed areas to prevent

recontamination (Amy, 2023). Food products must also be free from pathogenic bacteria such as *Salmonella* spp., with a maximum permissible limit of negative detection in 25 g of sample, according to Indonesian National Standards (SNI) and BPOM Regulation No. 3 (2026). However, hygiene practices in some campus canteens remain inadequate. Poor sanitation during peanut sauce preparation and the presence of flies as contamination vectors increase the risk of bacterial contamination. Consequently, several canteens have not fully met healthy canteen standards, raising concerns about food safety and public health.

A healthy canteen is defined as a food service facility that meets standards of hygiene, sanitation, and food safety. Food sold must be safe for consumption, both in terms of chemical content and the absence of pathogenic bacteria. Key criteria include providing safe, nutritionally balanced food free from hazardous substances, as regulated by the Indonesian Ministry of Health and the National Agency of Drug and Food Control (BPOM). These standards involve proper cooking, absence of rancidity, and clear nutritional labeling. Supporting facilities include handwashing stations, proper dishwashing areas with running water, safe storage systems, and adequate distance from waste disposal sites to prevent contamination (Ningsih & Khasanah, 2026).

Despite its importance, studies on the microbiological quality of ready-to-eat foods, particularly gado-gado, in the Tadulako University environment remain limited. Previous research has focused on processed or street foods, with little attention to traditional campus foods. Data on *Salmonella* spp. contamination in *gado-gado* are still lacking.

Therefore, this study provides insights by specifically examining the presence of *Salmonella* spp. in *gado-gado* sold in campus canteens, thereby contributing to the understanding of food safety risks in higher education environments and supporting efforts to improve healthy canteen standards.

MATERIALS AND METHODS

This study employed a descriptive exploratory design aimed at identifying the presence of *Salmonella* spp. in *gado-gado* sold within the campus area of Tadulako University. The research was conducted in the Biology Education Laboratory. The samples consisted of gado-gado obtained from five different campus canteens. Sampling was performed using a random sampling technique. Each sample was collected in ready-to-eat condition and immediately analyzed in the laboratory.

The equipment used in this study included beakers, Erlenmeyer flasks, test tubes, Durham tubes, test tube racks, Petri dishes, a Bunsen burner, spatulas, inoculating loops, micropipettes, an autoclave, an incubator, an analytical balance, a hot plate, glass slides, a microscope, and a digital camera. The materials used included gado-gado samples, Nutrient Agar (NA), Salmonella–Shigella Agar (SSA), Triple Sugar Iron Agar (TSIA), distilled water (aquades), and reagents for Gram staining, namely crystal violet, iodine solution, alcohol, and safranin.

Procedures

Sterilization of Equipment and Materials

All glassware, including test tubes, Petri dishes, and Erlenmeyer flasks, was wrapped in heat-resistant paper and sterilized using an autoclave at 121°C for 15 minutes. Culture media were also sterilized by autoclaving prior to use.

Sample Preparation and Serial Dilution

Gado-gado samples were weighed (10 g) and aseptically transferred into 90 mL of sterile distilled water to obtain a 10^{-1} dilution. Serial dilutions were subsequently performed up to 10^{-3}.

Most Probable Number (MPN) Test

A volume of 1 mL from each dilution level was inoculated into tubes containing Lactose Broth supplemented with Durham tubes. The tubes were incubated at 37°C for 24 hours. A positive result was indicated by gas formation in the Durham tubes and turbidity of the medium. The MPN value was determined based on the combination of positive tubes using a standard MPN table.

Total Plate Count (TPC)

One milliliter of each diluted sample was transferred into sterile Petri dishes, followed by the addition of molten Nutrient Agar using the pour plate method. After solidification, the plates were incubated at 37°C for 24 hours. The number of colonies formed was counted and expressed as colony-forming units per milliliter (CFU/ml).

Isolation on Selective Medium

Colonies grown on Nutrient Agar were streaked onto Salmonella–Shigella Agar (SSA) using the streak plate method. The plates were incubated at 37°C for 24–48 hours. Presumptive *Salmonella* spp. colonies were identified based on their morphological characteristics.

Gram Staining

Bacterial colonies were picked using a sterile inoculating loop, smeared onto a glass slide, and heat-fixed. The smear was stained with crystal violet for 1 minute and rinsed with water. Iodine solution was then applied for 1 minute, followed by rinsing and decolorization with 96% ethanol for 30 seconds. Subsequently, safranin was applied for 1–2 minutes. The slide was rinsed, air-dried, and observed under a microscope at 1000× magnification.

Biochemical Test (TSIA)
Bacterial colonies were inoculated into Triple Sugar Iron Agar (TSIA) using the stab-and-streak method. The medium was incubated at 37°C for 24 hours. Observations were made based on color changes in the slant and butt, as well as gas and H2S production, which served as indicators of bacterial biochemical activity.

RESULTS AND DISCUSSION

Presumptive Test Results

The first stage of the Most Probable Number (MPN) method involved a presumptive test to detect the presence of bacterial colonies through lactose fermentation in Lactose Broth medium. In this study, a 3-3-3 tube series arrangement was applied, followed by incubation at 37 °C for 24 hours. *Gado-gado* samples collected from five canteens in the vicinity of Tadulako University showed positive indications of bacterial presence. Positive results were indicated by gas formation in the Durham tubes, as well as turbidity and/or color changes in the Lactose Broth medium (Figure 1).

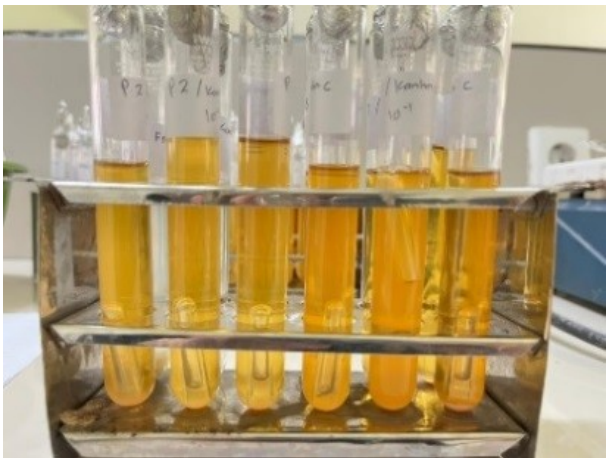


Figure 1. Color change in Lactose Broth medium after incubation for 24 hours, indicating bacterial metabolic activity

All five samples tested in Lactose Broth showed positive results at each dilution level, as indicated by the presence of gas formation and turbidity in the medium. The positive results were subsequently quantified using the Most Probable Number (MPN) table (Table 1).

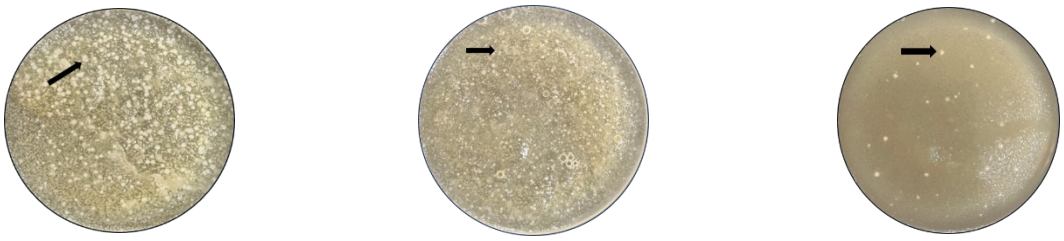
Table 1. Most Probable Number (MPN) of Bacterial Colonies in Lactose Broth Medium.

Sample (Gado-Gado)	Dilution			Most Probable Number (mL sampel)
	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 showed variation across dilution levels (10^{-1} to 10^{-3}) among the five sampling points in the canteens of Tadulako University. Canteen E exhibited the highest MPN value, reaching 150 × 10^2 MPN/mL sample, indicating a higher level of bacterial contamination compared to the other samples. Canteen C showed an intermediate value of 35 × 10^2 MPN/mL sample, while Canteens A, B, and D had the lowest MPN values, each recorded at 20 × 10^2 MPN/mL sample.

Total Plate Count (TPC) Results

Samples that showed positive results in the presumptive test using Lactose Broth were subsequently subcultured onto Nutrient Agar to determine the total number of bacterial colonies. The growth of bacterial colonies on Nutrient Agar resulted in visible colony formation (Figure 2).



(a) (b) (c)

Figure 2. Bacterial colonies on Nutrient Agar: (a) dilution 10^{-1}, (b) dilution 10^{-2}, and (c) dilution 10^{-3}.

The results of bacterial colony counts grown on Nutrient Agar using the Total Plate Count (TPC) method at dilution levels of 10^{-1} to 10^{-3} are presented in (Table 2).

Table 2. Total Plate Count (TPC) of Bacterial Colonies in Gado-Gado Samples.

Sample (Gado-Gado)	Dilution			Total Plate Count (mL sampel)
	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 showed variation in bacterial colony counts across dilution levels (10^{-1} to 10^{-3}) among the five sampling points in the canteens of Tadulako University. Canteen D exhibited the highest TPC value, reaching 537 × 10^2 CFU/mL, indicating a higher level of bacterial contamination compared to the other samples. This may be associated with suboptimal sanitation practices, such as inadequate washing of utensils, improper food storage (e.g., exposure to open environments), or humid environmental conditions that favor bacterial growth. In contrast, Canteen C showed the lowest bacterial colony count, with a TPC value of 215 × 10^2 CFU/mL. This lower contamination level may be attributed to better hygiene practices, effective waste management to prevent vector transmission, and stricter water quality control, thereby reducing the risk of bacterial contamination compared to the other sampling points.

Confirmatory Test on Selective Medium

The results of bacterial inoculation on selective Salmonella–Shigella Agar (SSA) showed colonies with distinct morphological characteristics. After incubation at 35°C for 24 hours, the colonies appeared circular, with a dark purplish to black coloration, moist and mucoid texture, irregular or serrated margins, and a convex elevation. The colonies formed on the selective medium are presented in (Figure 3).

Figure 3. Bacterial colonies on Salmonella–Shigella Agar.

Based on the observations, the colonies grown on SSA indicate that this medium supports the growth of *Salmonella* spp., which typically produce circular colonies with slightly raised surfaces and relatively small size. Some colonies exhibited black centers, which are indicative of hydrogen sulfide (H2S) production. These characteristics are consistent with the typical morphology of *Salmonella* spp. on SSA.

Gram Staining

Gram staining was performed to determine the bacterial group present in the samples, specifically to differentiate between Gram-negative and Gram-positive bacteria. Observations were carried out under a microscope at 1000× magnification (oil immersion). The morphological characteristics of the bacterial cells observed in the samples are presented in (Figure 4).

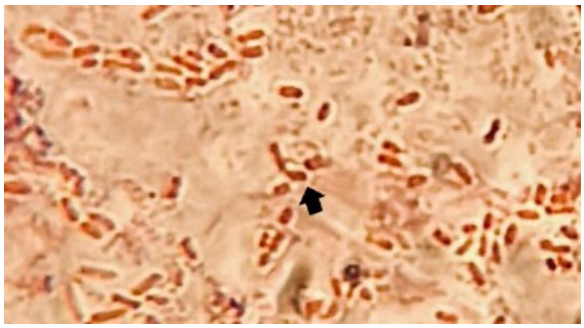


Figure 4. *Salmonella* spp. cells observed after Gram staining.

Biochemical Test Using Triple Sugar Iron Agar (TSIA)

Bacterial colonies grown on the selective medium were picked using a sterile inoculating loop and inoculated onto Triple Sugar Iron Agar (TSIA) to observe biochemical reactions characteristic of the bacterial isolates. The results of the biochemical analysis are presented in (Table 3).

Table 3. Biochemical Reaction Results on Triple Sugar Iron Agar (TSIA).

Bacteria Sample	Medium *Triple Sugar Iron Agar*			H_2S
	Butt	Slant	Gas	
Salmonella spp.	A	B	-	+

Notes:
A = Acid (yellow color of the medium)
B = Alkaline (red color of the medium)
H_2S = Black precipitate at the bottom of the tube
Gas = Presence of gas bubbles
\+ = Positive (gas and/or H2S production)
\- = Negative (no gas or H2S production)

Discussion

The analysis of bacterial contamination in *gado-gado* using the Most Probable Number (MPN) method with Lactose Broth yielded an average value of 4,900 CFU/mL of sample. This result is consistent with the findings of (Kurahman et al, 2022), who reported that most ready-to-eat foods in campus environments exhibit MPN values exceeding acceptable standards due to inadequate hygiene and sanitation practices. The high MPN values observed in this study indicate significant bacterial contamination in gado-gado samples.

According to the Indonesian National Standard (SNI No. 7388 of 2009), the maximum permissible limit for microbial contamination in processed food products is 1 × 10^1 (10 CFU/mL) (Zelpina et al, 2020). Therefore, all five *gado-gado* samples exceeded the established threshold, indicating that they do not meet food safety standards. These findings suggest that the tested *gado-gado* samples have a high level of bacterial contamination and are unsafe for consumption (Nurbaeti & Akbar, 2026). Consequently, it is essential to implement proper hygiene and sanitation practices throughout food processing, storage, and serving stages to minimize the risk of bacterial contamination (Dea et al, 2025).

The Total Plate Count (TPC) analysis of gado-gado samples showed an average value of 35,800 CFU/mL. Based on the Indonesian food safety regulation issued by the National Agency of Drug and Food Control (BPOM Regulation No. 3 of 2026), the maximum permissible limit for microbial contamination in processed food is 10^4 CFU/mL (BPOM, 2026). These findings indicate that the microbiological quality of gado-gado is strongly influenced by hygiene and sanitation practices during processing, storage, and serving. This result is supported by previous studies, which report that high TPC values in street foods are commonly associated with environmental contamination, inadequate sanitation of equipment, and poor food handling practices. Given that the TPC values of all tested samples exceeded the permissible limit, it can be concluded that the *gado-gado* samples do not meet microbiological safety standards (Stephani et al, 2025).

The results of bacterial cultivation on selective media showed colonies with circular morphology, dark purplish to black coloration, mucoid and moist texture, irregular margins, and convex elevation after incubation at 35°C for 24 hours (Solikhah et al, 2021). These characteristics are consistent with the findings of (Putri et al, 2023), who reported that *Salmonella* spp. colonies on Salmonella–Shigella Agar (SSA) typically appear pale with black centers due to hydrogen sulfide (H2S) production. The use of SSA, a selective medium for Salmonella and Shigella, further supports the identification of these bacteria (Harvianti et al, 2025). Overall, this study indicates that all *gado-gado* samples collected from canteens at Tadulako University were contaminated with *Salmonella* spp. This finding is consistent with previous studies reporting that bacterial colonies grown on SSA exhibit circular shapes, slightly convex surfaces, small size, and occasionally black centers due to H2S precipitation (Sinaga et al, 2021).

Gram staining was performed to determine the bacterial group present in the samples, specifically to distinguish between Gram-negative and Gram-positive bacteria. In this study, Gram staining of *Salmonella* spp. confirmed that the bacteria belong to the Gram-negative rod-shaped (bacilli) group. Microscopically, the cells appeared pink to red after staining, which is characteristic of Gram-negative bacteria. This occurs because their cell walls contain a thin peptidoglycan layer and a high lipid content, preventing retention of the primary crystal violet–iodine complex during the decolorization step with alcohol, and allowing uptake of the counterstain (safranin). This staining method is essential as an initial step in bacterial identification, enabling differentiation of *Salmonella* spp. from Gram-positive bacteria, which appear purple under the microscope. Furthermore, Gram staining supports the results of isolation and biochemical testing, strengthening the identification of the isolates as *Salmonella* spp. (Ismail et al, 2023).

Triple Sugar Iron Agar (TSIA) is a solid medium widely regarded as a standard method for the biochemical identification of *Salmonella* spp., as recommended in SNI 01-2332.2-2006 for microbiological testing of *Salmonella* in food products. On TSIA medium, positive results for *Salmonella* spp. are typically characterized by a red slant (alkaline reaction) and a yellow butt (acid reaction), indicating glucose fermentation without lactose and sucrose fermentation. In addition, gas production and hydrogen sulfide (H2S) formation may occur, with H2S indicated by black precipitate formation in the medium. In this study, incubation was carried out at 37°C for 48 hours. The observed changes—namely a red slant, yellow butt, and black precipitate—indicate positive biochemical reactions consistent with *Salmonella* spp. These findings are in agreement with previous studies reporting that *Salmonella* spp. exhibit an alkaline slant, acid butt, and H2S production on TSIA medium (Wibisono, 2017; Midorikawa et al, 2014). The interpretation of TSIA results is based on color changes in the medium, where a yellow color indicates acidic conditions, while a red color indicates alkaline conditions. A yellow butt indicates acid production, whereas a red slant indicates alkaline conditions. These observations are consistent with previous findings (Wijada, 2025).

The contamination of *Salmonella* spp. in *gado-gado* sold in canteens at Tadulako University indicates that hygiene and food handling practices in the preparation of this dish are inadequate. This finding is consistent with Anita et al. (2021), who reported that *Salmonella* contamination in ready-to-eat foods is commonly associated with poor hygiene practices and inadequate environmental sanitation. Studies conducted in various settings, including campus areas, have shown that up to 50% of *gado-gado* samples were positive for *Salmonella*, primarily due to insufficient cooking of vegetables and poor sanitation of food stalls. Health is considered a key factor influencing the quality of human resources; therefore, it requires serious attention. Maintaining good health supports daily activities and enhances productivity (Muldiawati et al, 2022). *Gado-gado*, as a widely consumed ready-to-eat food in Central Sulawesi—particularly around Tadulako University—has a high potential to cause foodborne illness. This is due to

conditions that favor microbial growth, especially the peanut sauce, which is rich in proteins and lipids, providing an ideal medium for pathogenic bacteria to proliferate. In addition, cross-contamination may occur through raw vegetables, contaminated utensils, washing water, and poor hygiene practices of food handlers (Anita et al, 2021). *Salmonella* contamination in food can arise from several factors, including poor environmental sanitation, prolonged food storage, and the use of inadequately protected equipment. Acute salmonellosis is characterized by symptoms such as fever, abdominal pain, diarrhea, and sometimes vomiting (Rizqoh & Ismuda, 2021).

A high number of bacterial colonies in *gado-gado* indicates poor sanitation during food processing, as these indicator bacteria often originate from human or animal fecal sources and reflect cross-contamination due to poor personal hygiene of food handlers, such as failure to wash hands, use of unclean utensils, or contaminated water (Annisa et al, 2024). The high total colony count exceeding the permissible limit set by the Indonesian National Standard (SNI No. 7388 of 2009; >10 CFU/mL) reflects unsanitary environmental conditions, including improper storage of raw vegetables in humid environments or insufficient cooking. These conditions may facilitate the presence of other pathogens such as *Salmonella* spp. These factors are interrelated, where poor sanitation and high microbial loads create an ideal environment for *Salmonella* spp. to survive and proliferate, thereby increasing the risk of foodborne illness among consumers (Rini, 2023).

To prevent bacterial contamination, food handlers should separate areas for raw and ready-to-eat food processing, use different cutting boards and knives, and avoid placing cooked food on surfaces previously used for raw materials. In general, the findings of this study are consistent with previous research indicating that ready-to-eat foods have a high risk of contamination by pathogenic bacteria if not handled under proper hygiene standards. Food handlers should wash their hands frequently using soap and running water before handling food, after using the toilet, and after handling money or contaminated objects. Raw vegetables should be thoroughly washed with clean running water, preferably through multiple rinses, to reduce microbial load on their surfaces (Irianti et al, 2022).

CONCLUSION

Based on the findings of this study, it can be concluded that *gado-gado* sold in the campus area of Tadulako University was presumptively contaminated with *Salmonella* spp. Furthermore, the microbiological quality of the tested samples did not meet the safety standards established by the Indonesian Ministry of Health, as indicated by MPN values reaching 4,900 MPN/mL, which exceed the permissible limit of 10 MPN/mL for processed food products. These findings highlight the need for improved sanitation and hygiene practices in food preparation, handling, and storage. In addition, regular microbiological monitoring and inspection by relevant authorities are essential to ensure food safety and protect public health.

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Conflicts of Interest: The authors declare that there are no conflicts of interest.

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