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PRESUMPTIVE IDENTIFICATION OF *SALMONELLA* SPP. IN MEATBALLS SOLD IN SELECTED SNACK AREAS OF GORONTALO CITY

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ABSTRACT

Food safety is an important public health concern, particularly for ready-to-eat meat products that may be exposed to contamination during processing, handling, storage, and serving. This study aimed to presumptively identify Salmonella spp. in meatballs sold in selected snack areas of Gorontalo City based on macroscopic, microscopic, and biochemical characteristics. A descriptive laboratory study with a cross-sectional approach was conducted in June 2024 at the Microbiology Laboratory of Universitas Bina Mandiri Gorontalo. Six meatball samples were collected purposively from four snack-selling areas and examined using Salmonella-Shigella Agar (SSA), Gram staining, and Triple Sugar Iron Agar (TSIA). The laboratory findings were analyzed descriptively by comparing colony morphology, Gram reaction, and biochemical characteristics with the typical characteristics of Salmonella. Five samples (A, B, C, E, and F) showed characteristics consistent with presumptive Salmonella spp., including Gram-negative rod-shaped cells and TSIA reactions characterized by a red slant and yellow butt, with hydrogen sulfide production and gas formation observed in most samples. Sample D showed no bacterial colony growth under the examination conditions. The findings indicate that most examined meatball samples showed bacterial characteristics consistent with presumptive Salmonella spp. Therefore, improved hygiene, sanitation, food protection, and microbiological monitoring are recommended. Confirmatory biochemical, serological, or molecular testing is needed to establish definitive identification.

Keywords: *Salmonella* spp.; meatballs; food safety; street-vended food; microbiological identification

INTRODUCTION

Food safety remains a major public health concern because food can serve as a vehicle for pathogenic microorganisms throughout the food chain, from production and processing to retail and consumption. Among foodborne pathogens, *Salmonella* spp. are of particular concern because their presence in food can lead to gastroenteritis and, in severe cases, systemic infection. Contamination may occur at multiple points in the food chain and can be facilitated by inadequate hygiene, improper handling, and cross-contamination [1][2][3].

Meat and meat-based products are particularly vulnerable to microbial contamination because their nutritional composition and moisture content can support bacterial survival and growth. *Salmonella* may be introduced during slaughter, processing, transportation, preparation, or retail handling, including through contaminated equipment and

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contact surfaces (Acuff, 2014; Nidaullah et al., 2016). Meatballs, which are widely consumed as an affordable ready-to-eat food in Indonesia, may therefore present a potential food safety concern when hygienic preparation, handling, storage, and serving practices are inadequate. The presence of *Salmonella* in meat products is particularly important because contaminated products may pose a direct risk to consumers (Da Costa et al., 2022).

Street-vended foods require particular attention because their safety is influenced not only by the quality of raw materials but also by environmental and handling conditions. Previous studies have reported the occurrence of *Salmonella* in street-vended foods and ready-to-eat meat products, with contamination associated with inadequate food-handling practices and environmental exposure (Anukampa et al., 2017; Aduah et al., 2021; Sharma & Mazumdar, 2014). Potential sources include food handlers, contaminated utensils and surfaces, raw materials, water, dust, insects, and inappropriate storage conditions. Cross-contamination can occur when food comes into contact with contaminated equipment or when hygiene practices are insufficient during preparation and serving (Carrasco et al., 2012). Consequently, maintaining appropriate hygiene and sanitation throughout food handling is essential for reducing the risk of foodborne pathogens.

Preliminary field observations in snack areas of Gorontalo City indicated several conditions that may facilitate microbial contamination of meatballs. Some vendors displayed meatballs in open containers, exposing the products to dust and the surrounding environment. The cleanliness of vending areas also varied, with differences in the availability of waste bins and the general cleanliness around food stalls. Interviews with several vendors further indicated that some handlers did not routinely use gloves during food preparation and relied mainly on handwashing. In addition, some vendors reported using meatballs obtained from other production sites, limiting direct observation of the processing conditions at the source. These observations do not establish the presence or source of *Salmonella*, but they indicate the need for microbiological assessment of ready-to-eat meatballs sold in these environments.

Although previous studies have examined *Salmonella* contamination in meat products and street-vended foods, evidence specifically concerning meatballs sold in snack areas of Gorontalo City remains limited. Local surveillance is important because contamination risks may vary according to vending environments, food-handling practices, and local sanitation conditions. Therefore, laboratory-based assessment is necessary to determine whether meatball samples show characteristics consistent with *Salmonella* contamination.

This study aimed to presumptively identify *Salmonella* spp. in meatballs sold in selected snack areas of Gorontalo City using macroscopic colony characteristics, Gram staining, and biochemical examination using Triple Sugar Iron Agar (TSIA). The findings are expected to provide preliminary microbiological evidence regarding the safety of meatballs sold in these areas and contribute to food hygiene and safety efforts in Gorontalo City.

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Literature Review

Salmonella spp.: Characteristics, Morphology, Growth, and Pathogenicity

Salmonella spp. are important foodborne pathogens that can contaminate a wide range of animal-derived foods, particularly meat and meat products. They are Gram-negative, rod-shaped bacteria belonging to the family Enterobacteriaceae and are generally capable of surviving under diverse environmental conditions. Their persistence throughout the food chain contributes to their importance as a food safety and public health concern (Tafida et al., 2013; Ljubojević Pelić et al., 2021). The ability of Salmonella to survive in food-processing and retail environments increases the possibility of transmission to humans through consumption of contaminated products.

Human infection with Salmonella is commonly associated with the consumption of contaminated food or water. The clinical manifestations may range from gastrointestinal symptoms to more severe systemic infections, depending on the serovar, bacterial load, and susceptibility of the host. The epidemiological significance of Salmonella in animal-derived foods is therefore closely related to its ability to enter the food chain and persist until consumption (Ljubojević Pelić et al., 2021; Oludairo et al., 2022).

Salmonella Contamination in Meat-Based Foods

Meat and meat products are recognized as important vehicles for *Salmonella* transmission because contamination may occur at several stages, including slaughter, processing, transportation, preparation, storage, and retail handling. Studies have reported the occurrence of Salmonella in retail beef and other meat products, demonstrating that contamination can persist beyond primary production and reach consumers through retail food products (Tafida et al., 2013). Similarly, the detection of Salmonella in ready-to-eat roasted meat indicates that inadequate handling and environmental exposure may contribute to contamination of products intended for direct consumption (Oludairo et al., 2022).

Cross-contamination is another important mechanism by which Salmonella can spread between raw materials, food-contact surfaces, utensils, and ready-to-eat foods. Differences in storage conditions and retail practices may further influence bacterial survival and persistence in meat products (Nidaullah et al., 2016). Consequently, contamination control requires attention throughout the food chain rather than focusing only on the initial source of the meat.

Hygiene and Sanitation in Street-Vended Foods

Street-vended foods provide affordable and accessible food for urban populations but may be exposed to several food safety hazards. The microbiological quality of these foods can be influenced by environmental conditions, food-handling practices, vendor knowledge, sanitation facilities, temperature control, and regulatory compliance. Previous studies have demonstrated that street-vended foods may contain pathogenic microorganisms when appropriate hygiene and sanitation practices are not consistently implemented (Jahan et al., 2018; Mwove et al., 2020).

Poor personal hygiene, contaminated utensils and surfaces, inadequate protection of food from

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environmental exposure, and inappropriate storage conditions can facilitate microbial contamination. A systematic review by Cataluna and Rukmini (2024) emphasized the importance of food hygiene and sanitation knowledge and practices among street food vendors in reducing food safety risks. Furthermore, compliance with food safety regulations and effective enforcement are important components of controlling microbiological hazards in street food vending environments (Alawode & Tabit, 2025).

Laboratory Identification of Salmonella spp.

The microbiological identification of *Salmonella* generally involves a sequence of isolation, characterization, and biochemical or molecular identification procedures. Selective and differential culture media are commonly used to facilitate the recovery and preliminary differentiation of suspected *Salmonella* colonies from other microorganisms. Salmonella-Shigella Agar (SSA), Xylose Lysine Deoxycholate (XLD), and other selective media have been used in microbiological investigations of Salmonella. Comparative studies have demonstrated differences in recovery performance among culture media, highlighting the importance of appropriate media selection and laboratory procedures (Yue et al., 2014).

Following colony growth, Gram staining can provide preliminary morphological information. Salmonella typically appears as Gram-negative rods under microscopic examination. However, Gram-negative rod morphology alone cannot establish the identity of Salmonella, because several other bacterial genera have similar morphological characteristics. Therefore, microscopic examination should be combined with additional biochemical or molecular tests.

Biochemical testing provides further evidence for the presumptive identification of Salmonella. Triple Sugar Iron Agar (TSIA) is commonly used to evaluate bacterial carbohydrate fermentation and hydrogen sulfide production. A typical reaction associated with Salmonella may include an alkaline slant, an acidic butt, and hydrogen sulfide production; however, TSIA results should be interpreted together with other identification characteristics rather than being regarded as definitive confirmation by themselves. Comparative evaluations of biochemical identification systems have demonstrated the importance of combining multiple biochemical characteristics for reliable identification of Salmonella and related bacteria (Yang et al., 2023).

Although conventional culture and biochemical methods remain important for microbiological investigation, they generally require longer processing times than molecular approaches. Molecular methods such as polymerase chain reaction (PCR) have therefore been developed to provide more rapid detection of Salmonella by targeting specific genetic markers (Camacho et al., 2008; Jawad & Al-Charrakh, 2016). Nevertheless, conventional culture-based approaches remain valuable, particularly in settings where molecular diagnostic facilities are limited and where preliminary isolation and characterization of suspected bacterial colonies are required.

Relevance to Meatball Safety

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The characteristics of Salmonella, the susceptibility of meat-based products to contamination, and the potential risks associated with street-vending environments provide an important basis for assessing the microbiological safety of meatballs. Meatballs are commonly prepared from animal-derived ingredients and may undergo multiple stages of handling before being sold to consumers. Consequently, contamination may occur through raw materials, food handlers, utensils, storage containers, or environmental exposure.

In the context of Gorontalo City, preliminary observations of meatball vending sites identified several conditions that may facilitate microbial contamination, including open food displays, variable environmental cleanliness, exposure to dust and traffic, and inconsistent use of protective gloves by food handlers. These observations should not be interpreted as direct evidence that such factors caused Salmonella contamination; rather, they represent potential risk conditions that justify laboratory-based assessment.

Based on the existing literature, laboratory examination is therefore necessary to provide preliminary evidence regarding the presence of bacterial characteristics consistent with Salmonella in meatballs sold in local snack areas. In this study, macroscopic colony characteristics, Gram staining, and biochemical examination using TSIA were used to support the presumptive identification of Salmonella spp. in meatball samples obtained from selected snack areas in Gorontalo City.

RESEARCH METHODS

Research Design

This study employed a descriptive laboratory research design with a cross-sectional approach. The study aimed to identify the presence or absence of *Salmonella* sp. in meatball snacks sold in several culinary areas of Gorontalo City. The identification of *Salmonella* sp. was performed through macroscopic, microscopic, and biochemical examinations. Macroscopic examination was conducted by observing the morphological characteristics of bacterial colonies, microscopic examination was performed using Gram staining, and biochemical identification was carried out using Triple Sugar Iron Agar (TSIA) medium.

The research was conducted in June 2024. Laboratory examinations were performed at the Microbiology Laboratory of Universitas Bina Mandiri Gorontalo.

Research Location and Sampling Sites

The samples were collected from several meatball snack-selling areas in Gorontalo City, namely:

1. Green Open Space (Ruang Terbuka Hijau/RTH);
2. Kalimadu Culinary Park;
3. Malioboro area; and
4. The area in front of Universitas Negeri Gorontalo (UNG).

These locations were selected purposively because they have a relatively high number of meatball vendors operating in open or roadside environments. Based on preliminary observations, several vendors were found to display meatball products in containers that were not consistently covered, potentially increasing the risk of exposure to environmental contaminants, including bacteria.

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Population and Sample

The population in this study consisted of meatball snacks sold by vendors at the selected sampling locations in Gorontalo City. Sampling was conducted using a purposive sampling technique, based on predetermined criteria relevant to the objectives of the study.

Based on preliminary observations, there were approximately 2–3 meatball vendors at the observed locations. A total of six meatball samples were collected for laboratory examination. Each sample was collected aseptically to minimize contamination during the sampling and transportation processes.

Data Sources

The data used in this study consisted of primary and secondary data.

1. Primary data were obtained directly from the results of laboratory examinations of meatball samples. The primary data included macroscopic, microscopic, and biochemical characteristics associated with the identification of *Salmonella* sp.
2. Secondary data were obtained from relevant scientific literature, including journal articles, books, and previous research related to food contamination and the identification of *Salmonella* sp. in food products.

Data Collection Techniques

Data were collected through observation, interviews, documentation, and laboratory examinations.

Observation

Observation was conducted at the sampling locations to identify the environmental conditions surrounding meatball vendors, including the way meatball products were stored and displayed. Observation was also used to determine the suitability of the sampling locations based on the predetermined criteria.

Interviews

Interviews were conducted with meatball vendors to obtain supporting information related to the handling, storage, and serving of meatball products. The interviews were conducted directly with the vendors using questions relevant to the research objectives.

Documentation

Documentation was conducted by collecting supporting materials related to the research process, including photographs of sampling activities, sample collection, laboratory procedures, and laboratory examination results. Documentation was used to support and clarify the findings obtained through observation, interviews, and laboratory examinations.

Laboratory Examination

Laboratory examination was the primary data collection technique used to determine the presence or absence of *Salmonella* sp. in the meatball samples. The laboratory examination consisted of three stages: pre-analytical, analytical, and post-analytical stages.

Laboratory Examination Procedures

Pre-Analytical Stage

The pre-analytical stage included preparation of equipment and materials, sample collection, sample transportation, and preparation of samples before examination.

The equipment used in this study included micropipettes, a meat blender, Petri dishes, test tubes, test tube racks, blue tips, analytical balances, Bunsen burners, beakers, Erlenmeyer flasks, inoculating

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loops, staining racks, hotplates, autoclaves, colony counters, and a Laminar Air Flow (LAF) cabinet.

The materials used included Salmonella-Shigella Agar (SSA) medium, Triple Sugar Iron Agar (TSIA) medium, sodium chloride (NaCl), distilled water, crystal violet, iodine solution, 95% ethanol, safranin, and meatball samples.

Sample Collection and Transportation

Meatball samples were collected aseptically using sterile plastic containers or bags. The researcher requested the vendor to place the meatball sample directly into the sterile container. The sampling process was conducted as close as possible to the original storage or serving container to minimize the possibility of contamination from the surrounding environment.

After collection, the samples were immediately placed in a cooler box containing ice packs and transported to the Microbiology Laboratory of Universitas Bina Mandiri Gorontalo for examination. The samples were processed as soon as possible after arrival at the laboratory.

Analytical Stage

The analytical stage consisted of equipment sterilization, sample preparation, sample dilution, preparation of SSA medium, inoculation of samples onto the medium, macroscopic examination, microscopic examination using Gram staining, and biochemical examination using TSIA medium.

Equipment Sterilization

Laboratory equipment requiring sterilization was prepared and sterilized using appropriate sterilization procedures. Equipment such as glassware was sterilized using an oven, while heat-sensitive materials or media were sterilized using an autoclave according to standard laboratory procedures.

Sample Preparation

Approximately 1 gram of each meatball sample was weighed using an analytical balance. The sample was then homogenized or crushed using a sterile meat blender until a uniform sample preparation was obtained. The prepared sample was subsequently subjected to dilution before inoculation onto the selective medium.

Preparation of SSA Medium

SSA medium was prepared according to the manufacturer's instructions. A total of 21.6 g of SSA medium was weighed for the required volume corresponding to the prepared Petri dishes. The medium was dissolved in distilled water in an Erlenmeyer flask and homogenized. The suspension was then heated using a hotplate until completely dissolved. The prepared medium was subsequently sterilized as required, allowed to cool to an appropriate temperature, and aseptically poured into sterile Petri dishes. The medium was allowed to solidify before use.

Inoculation of Samples

Following sample dilution, approximately 1 mL of the diluted sample was inoculated onto the prepared SSA medium using aseptic techniques. The inoculated medium was subsequently incubated at 37°C for 24 hours. After incubation, the growth of bacterial colonies was observed and their macroscopic characteristics were recorded.

Macroscopic Examination

Macroscopic examination was performed by observing the morphological

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characteristics of bacterial colonies growing on SSA medium. The characteristics observed included colony color, shape, margin, and elevation. Colonies showing characteristics suggestive of *Salmonella* sp. were selected for further microscopic and biochemical examination.

Microscopic Examination Using Gram Staining

Microscopic identification was performed using the Gram staining method. A bacterial smear was prepared aseptically by transferring a selected colony onto a clean glass slide using a sterile inoculating loop. The smear was allowed to air-dry and was subsequently heat-fixed.

The smear was then stained sequentially with crystal violet for approximately 1 minute, followed by rinsing with water. Gram's iodine was then applied for approximately 1 minute and the preparation was rinsed. Decolorization was performed using 95% ethanol for an appropriate duration while avoiding excessive decolorization. The smear was rinsed with water and counterstained with safranin for approximately 45 seconds. The preparation was subsequently rinsed, dried, and examined microscopically using the oil-immersion objective.

The microscopic characteristics considered indicative of *Salmonella* sp. included Gram-negative, rod-shaped bacterial cells.

Biochemical Examination Using TSIA Medium

Biochemical identification was performed using Triple Sugar Iron Agar (TSIA) medium. Colonies suspected of being *Salmonella* sp. were taken aseptically using a sterile inoculating loop and inoculated into the TSIA medium by stabbing the butt and streaking the slant surface.

The inoculated TSIA medium was incubated at 37°C for 24–48 hours. The biochemical reaction was subsequently observed based on changes in the color of the slant and butt, gas production, and hydrogen sulfide (H_2S) production. A reaction suggestive of *Salmonella* sp. was characterized by an alkaline slant and acidic butt (red/yellow), with possible H_2S production indicated by blackening of the medium and possible gas formation.

Post-Analytical Stage

The post-analytical stage consisted of reading, recording, and interpreting the laboratory examination results. The results obtained from macroscopic, microscopic, and biochemical examinations were compared with the characteristic identification criteria for *Salmonella* sp.

The identification was based on the combination of the following characteristics:

1. Macroscopic characteristics: colony morphology, including color, shape, margin, and elevation, observed on SSA medium;
2. Microscopic characteristics: Gram-negative, rod-shaped bacterial cells observed following Gram staining; and
3. Biochemical characteristics: TSIA reactions, including changes in the slant and butt, H_2S production, and gas formation.

The results were subsequently presented descriptively to determine whether the examined meatball samples showed characteristics consistent with the presence of *Salmonella* sp.

Data Analysis

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The laboratory examination results were analyzed descriptively. The results of macroscopic, microscopic, and biochemical examinations for each meatball sample were recorded and compared with the established characteristics of *Salmonella* sp. The findings were then presented in tabular and narrative forms to describe the presence or absence of bacterial characteristics consistent with *Salmonella* sp. in the examined meatball samples.

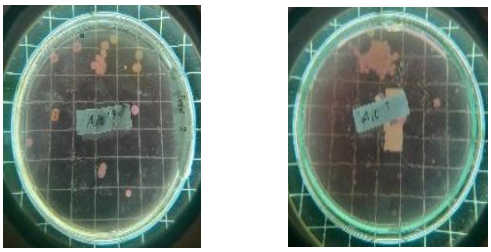
RESEARCH RESULTS

The laboratory examination of six meatball samples collected from several snack-selling areas in Gorontalo City was conducted through three stages of bacterial identification: macroscopic examination, microscopic examination, and biochemical testing. Macroscopic examination was performed by observing colony morphology on Salmonella-Shigella Agar (SSA) medium, microscopic examination was conducted using Gram staining, and biochemical identification was performed using Triple Sugar Iron Agar (TSIA) medium. The results obtained from each examination are presented below.

Macroscopic Examination

Macroscopic examination was conducted by observing the morphological characteristics of bacterial colonies growing on SSA medium after incubation. The observed characteristics included colony shape, color, margin, and elevation. The examination was performed on samples at 10^{-4} and 10^{-5} dilutions.

1. **Sample A**

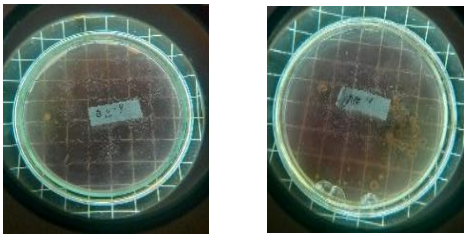


(a) **(b)**

Figure 1. Macroscopic characteristics of Sample A on SSA medium: (a) 10^{-4} dilution and (b) 10^{-5} dilution.

At the 10^{-4} dilution, Sample A showed colonies with predominantly round shapes, pink to yellow coloration, and some colonies with black centers. The colonies had entire margins and convex elevations. At the 10^{-5} dilution, the colonies were predominantly round, pink in color, with entire margins and convex elevations.

2. **Sample B**



(a) **(b)**

Figure 2. Macroscopic characteristics of Sample B on SSA medium: (a) 10^{-4} dilution and (b) 10^{-5} dilution.

At the 10^{-4} dilution, Sample B showed predominantly round colonies with yellow coloration, entire margins, and convex elevations. At the 10^{-5} dilution, both round and oval colonies were observed, with pink coloration and some colonies showing black pigmentation in the center. The colonies exhibited entire margins and convex elevations.

3. **Sample** C

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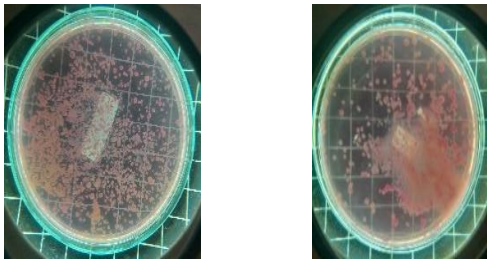
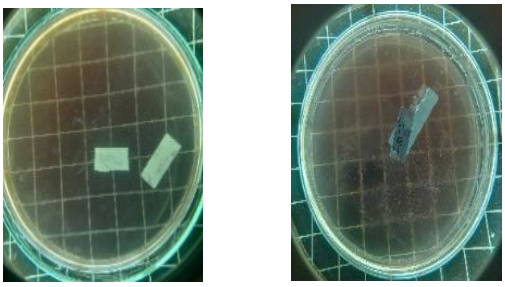


(a) **(b)**

Figure 3. Macroscopic characteristics of Sample C on SSA medium: (a) 10^{-4} dilution and (b) 10^{-5} dilution.

At the 10^{-4} dilution, Sample C showed round colonies with pink to yellow coloration, entire margins, and convex elevations. At the 10^{-5} dilution, round and oval colonies were observed, with pink to white coloration. The colonies had entire margins and convex elevations.

4. **Sample** D

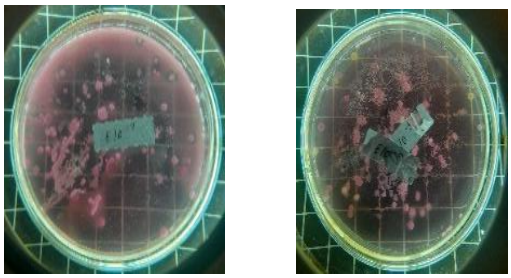


(a) **(b)**

Figure 4. Macroscopic characteristics of Sample D on SSA medium: (a) 10^{-4} dilution and (b) 10^{-5} dilution.

No bacterial colonies were observed on the SSA medium inoculated with Sample D at either the 10^{-4} or 10^{-5} dilution.

5. **Sample** E



(a) **(b)**

Figure 5. Macroscopic characteristics of Sample E on SSA medium: (a) 10^{-4} dilution and (b) 10^{-5} dilution.

At the 10^{-4} dilution, Sample E showed spherical or round colonies with transparent pink coloration, entire margins, and convex elevations. At the 10^{-5} dilution, round and oval colonies were observed with pink, white, and yellow coloration. Some colonies exhibited black pigmentation in the center. The colonies had entire margins and convex elevations.

6. **Sample** F

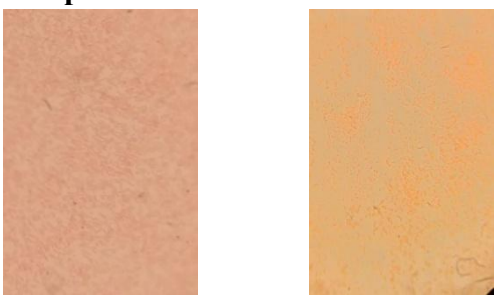
Figure 6. Macroscopic characteristics of Sample F on SSA medium: (a) 10^{-4} dilution and (b) 10^{-5} dilution.

At the 10^{-4} dilution, Sample F showed spherical colonies with pink to yellow coloration, entire margins, and convex elevations. At the 10^{-5} dilution, irregularly round colonies with pink to yellow coloration were observed. The colonies exhibited entire margins and convex elevations.

Microscopic Examination

Microscopic examination was performed using Gram staining to determine the cellular morphology and Gram reaction of the bacteria isolated from the meatball samples. The examination focused on bacterial shape and staining characteristics.

1. **Sample A**



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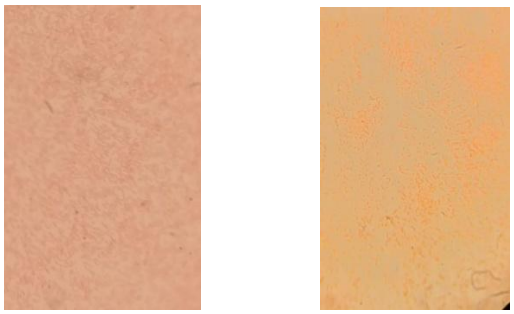
(a) (b)

Figure 7. Microscopic characteristics of Sample A after Gram staining: (a) 10^{-4} dilution and (b) 10^{-5} dilution.

Microscopic examination of Sample A at both the 10^{-4} and 10^{-5} dilutions showed rod-shaped bacterial cells (bacilli) with a Gram-negative reaction.

2. **Sample B**



(a) (b)

Figure 8. Microscopic characteristics of Sample B after Gram staining: (a) 10^{-4} dilution and (b) 10^{-5} dilution.

Sample B at both dilutions showed rod-shaped bacterial cells with a Gram-negative reaction.

3. **Sample C**

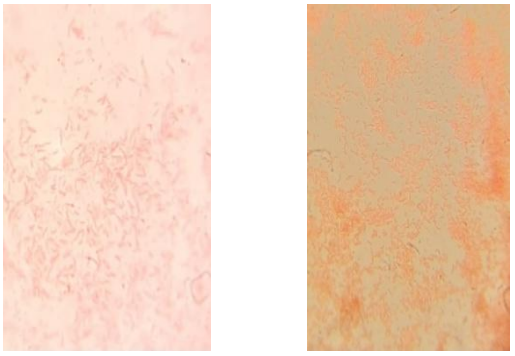
Figure 9. Microscopic characteristics of Sample C after Gram staining: (a) 10^{-4} dilution and (b) 10^{-5} dilution.

Microscopic examination of Sample C at the 10^{-4} and 10^{-5} dilutions revealed Gram-negative, rod-shaped bacterial cells.

4. **Sample D**

No colonies were observed in Sample D at either dilution; therefore, microscopic examination was not performed.

5. **Sample E**

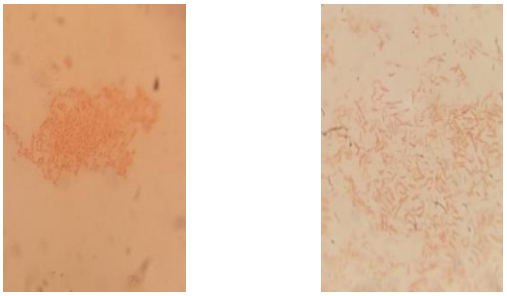


(a) (b)

Figure 10. Microscopic characteristics of Sample E after Gram staining: (a) 10^{-4} dilution and (b) 10^{-5} dilution.

Sample E at both the 10^{-4} and 10^{-5} dilutions showed rod-shaped bacterial cells with a Gram-negative reaction.

6. **Sample F**



(a) (b)

Figure 11. Microscopic characteristics of Sample F after Gram staining: (a) 10^{-4} dilution and (b) 10^{-5} dilution.

Microscopic examination of Sample F at both dilutions showed rod-shaped bacterial cells with a Gram-negative reaction.

Biochemical Examination

Biochemical identification was performed using Triple Sugar Iron Agar (TSIA) medium. The observed reactions included changes in the color of the slant and butt, gas production, and hydrogen sulfide (H_2S) production. The biochemical reactions of each sample are presented below.

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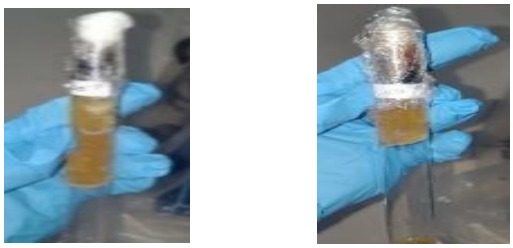
1. **Sample A**

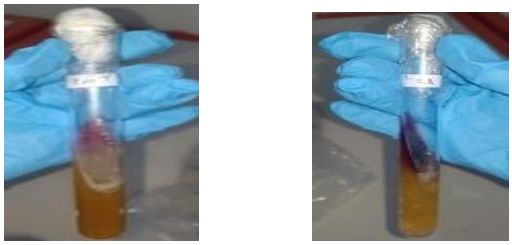


(a) **(b)**

Figure 12. TSIA reaction of Sample A: (a) 10^{-4} dilution and (b) 10^{-5} dilution.

At both the 10^{-4} and 10^{-5} dilutions, Sample A produced an alkaline slant and an acidic butt, indicated by a red slant and yellow butt. Blackening of the medium was also observed, indicating H_2S production. The medium showed evidence of gas production.

2. **Sample B**



(a) **(b)**

Figure 13. TSIA reaction of Sample B: (a) 10^{-4} dilution and (b) 10^{-5} dilution.

At the 10^{-4} dilution, Sample B produced a red slant and yellow butt without blackening, indicating no detectable H_2S production. No visible gas production was observed. At the 10^{-5} dilution, the medium showed a red slant and yellow butt accompanied by blackening, indicating H_2S production. Gas production was also observed based on the lifting or displacement of the medium.

3. **Sample C**

Figure 14. TSIA reaction of Sample C: (a) 10^{-4} dilution and (b) 10^{-5} dilution.

At both dilutions, Sample C showed a red slant and yellow butt, accompanied by blackening of the medium, indicating H_2S production. The medium also showed evidence of gas production.

4. **Sample E**

Figure 15. TSIA reaction of Sample E: (a) 10^{-4} dilution and (b) 10^{-5} dilution.

At both the 10^{-4} and 10^{-5} dilutions, Sample E produced a red slant and yellow butt, with blackening of the medium indicating H_2S production. Gas production was also observed based on the displacement or lifting of the medium.

5. **Sample F**

Figure 16. TSIA reaction of Sample F: (a) 10^{-4} dilution and (b) 10^{-5} dilution.

At both dilutions, Sample F produced a red slant and yellow butt, accompanied by blackening of the medium, indicating H_2S production. The medium also showed evidence of gas production.

Summary of Laboratory Examination Results

The results of the macroscopic, microscopic, and biochemical examinations are summarized in Table 1.

Table 1. Summary of the Identification Characteristics of Salmonella sp. in Meatball Samples

Sample	Macroscopic Examination	Microscopic Examination	TSIA Reaction	Interpretation
A	Round colonies, pink/yellow coloration	Gram-negative bacilli	Red slant/yellow butt, H_2S	Consistent with presumptive

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Sample	Macroscopic Examination	Microscopic Examination	TSIA Reaction	Interpretation
	, some black-centered colonies, convex elevation		(+), gas (+)	Salmonella sp.
B	Round/oval colonies, pink/yellow coloration, some black-centered colonies	Gram-negative bacilli	Red slant/yellow butt; H_2S variable; gas (+) at 10^{-5}	Consistent with presumptive Salmonella sp.
C	Round/oval colonies, pink-white/yellow coloration, convex elevation	Gram-negative bacilli	Red slant/yellow butt, H_2S (+), gas (+)	Consistent with presumptive Salmonella sp.
D	No colonies observed	Not examined	Not examined	No bacterial growth detected
E	Round/oval colonies, pink/white/yellow coloration, some black-centered colonies	Gram-negative bacilli	Red slant/yellow butt, H_2S (+), gas (+)	Consistent with presumptive Salmonella sp.
F	Spherical/irregular round colonies, pink-yellow coloration	Gram-negative bacilli	Red slant/yellow butt, H_2S (+), gas (+)	Consistent with presumptive Salmonella sp.

DISCUSSION

Presumptive Identification of Salmonella spp. in Meatball Samples

The present study found that five of the six meatball samples examined (Samples A, B, C, E, and F) exhibited bacterial characteristics consistent with the presumptive identification of Salmonella spp., based on the combination of macroscopic, microscopic, and biochemical examinations. In contrast, Sample D showed no bacterial colony growth on Salmonella-Shigella Agar (SSA) medium at either the 10^{-4} or 10^{-5} dilution. The findings indicate that bacterial contamination was detected in most of the examined meatball samples, although the results should be interpreted as presumptive rather than definitive identification of Salmonella spp.

The macroscopic examination showed variations in colony morphology among Samples A, B, C, E, and F. Several samples exhibited round or oval colonies with pink, yellow, or white coloration, while some colonies showed black pigmentation in the center. The presence of black-centered colonies is particularly relevant because it may indicate hydrogen sulfide (H_2S) production, which is one of the characteristics that can be associated with Salmonella on differential media. However, colony morphology alone is insufficient to establish the identity of Salmonella, because other microorganisms may produce similar colony characteristics. Therefore, the macroscopic findings in this study were used as an initial screening characteristic and were subsequently supported by microscopic and biochemical examinations.

The microscopic examination further demonstrated that isolates obtained from Samples A, B, C, E, and F consisted of Gram-negative, rod-shaped bacterial

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cells. This finding is compatible with the general morphological characteristics of Salmonella, which is a Gram-negative bacillus belonging to the family Enterobacteriaceae [4][5]. Nevertheless, Gram-negative rod morphology is not specific to Salmonella, as several other members of Enterobacteriaceae and other Gram-negative bacteria may exhibit similar microscopic characteristics. Therefore, the microscopic findings strengthen the presumptive identification but cannot independently confirm the presence of Salmonella spp.

The biochemical examination using TSIA provided additional evidence for the presumptive identification. Samples A, C, E, and F showed a red slant and yellow butt accompanied by blackening of the medium and gas production. This reaction indicates an alkaline slant and acidic butt, with H_2S production and gas formation. Such a biochemical pattern is compatible with the characteristics commonly associated with Salmonella. Yang et al. [6], noted that biochemical characteristics are important in differentiating Salmonella and related bacterial groups, although individual biochemical reactions should be interpreted together with other identification tests.

An interesting finding was observed in Sample B, in which the TSIA reaction differed between the two dilutions. At the 10^{-4} dilution, the medium showed a red slant and yellow butt without detectable H_2S production or gas formation, whereas the 10^{-5} dilution showed blackening and evidence of gas production. This difference may reflect variation in the bacterial population or the composition of colonies transferred from the respective dilution plates. It also demonstrates that biochemical reactions may vary depending on the isolate selected for testing. Therefore, the result of Sample B should be interpreted cautiously and in conjunction with its macroscopic and microscopic characteristics rather than based on the TSIA reaction alone.

The overall finding that five out of six samples showed characteristics consistent with presumptive Salmonella spp. is important from a food safety perspective. Meatballs are ready-to-eat meat-based products and are commonly consumed directly after preparation or serving. Meat and meat products are susceptible to contamination at multiple stages, including processing, transportation, storage, preparation, and retail handling [7][8]. Consequently, the detection of bacterial characteristics consistent with Salmonella in five samples suggests that microbiological hazards may occur in the examined vending environment.

The findings are also consistent with previous studies reporting the occurrence of Salmonella in meat products and ready-to-eat foods. Tafida et al. [4], reported the occurrence of Salmonella in retail meat products, demonstrating that contamination may persist until the retail stage. Similarly, Oludairo et al. [9], identified Salmonella in ready-to-eat roasted meat, indicating that foods intended for direct consumption may remain vulnerable to contamination when handling and environmental controls are inadequate. These findings support the relevance of microbiological monitoring of ready-to-eat meat products, including meatballs sold in informal or street-vending environments.

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Potential Food Safety Risks Associated with Meatball Vending Conditions

The laboratory findings should also be considered in relation to the environmental and food-handling conditions observed at the sampling locations. Preliminary observations in the present study identified several conditions that could potentially facilitate microbial contamination, including open food containers, variable environmental cleanliness, exposure to dust and surrounding activities, and inconsistent use of protective gloves by food handlers. Some vendors also obtained meatballs from external production sites, meaning that the researchers could not directly assess the hygienic conditions during the earlier stages of production.

These conditions are relevant because contamination of ready-to-eat foods may occur through several routes. Food handlers, utensils, food-contact surfaces, water, raw materials, dust, insects, and inappropriate storage conditions can all contribute to microbial contamination [3]. In particular, foods that are displayed in open containers may have greater exposure to environmental contaminants than foods that are adequately protected during storage and serving.

The observed conditions do not, however, establish a direct causal relationship between specific vending practices and the presumptive detection of Salmonella in the samples. The present study did not quantitatively assess the hygiene practices of individual vendors, environmental microbial loads, water quality, or contamination at the production source. Therefore, the observed vending conditions should be regarded as potential risk factors rather than confirmed sources of contamination. This distinction is important because Salmonella contamination can occur at multiple points along the food chain, including before the product reaches the vendor.

Previous studies have similarly emphasized the importance of hygiene and sanitation in street-vended foods. Jahan et al. [10], and Mwove et al. [11]. reported that the microbiological quality of street foods can be influenced by food-handling practices, sanitation facilities, environmental conditions, and storage practices. Cataluna and Rukmini [12], further emphasized the role of food hygiene and sanitation knowledge and practices among street food vendors in reducing food safety risks. Thus, the findings of the present study reinforce the importance of applying appropriate hygiene and sanitation measures during the handling and serving of meatball products.

The use of appropriate food protection measures is particularly important because meatballs are generally consumed without additional processing by consumers. Once contamination occurs after cooking, subsequent handling may allow microorganisms to remain on the product until consumption. Therefore, maintaining clean utensils, preventing contact between ready-to-eat food and potentially contaminated surfaces, protecting food from environmental exposure, and implementing appropriate personal hygiene practices are important measures for reducing potential foodborne hazards.

Implications for Food Safety and Limitations of Laboratory Identification

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The detection of presumptive Salmonella characteristics in five of six samples has practical implications for food safety in the examined snack areas. Although the study involved a relatively small number of samples, the findings provide preliminary microbiological evidence that meatballs sold in these locations may be exposed to bacterial contamination. This supports the need for increased attention to food hygiene practices among vendors and continued microbiological surveillance of ready-to-eat meat products.

From a laboratory perspective, the combination of SSA culture, Gram staining, and TSIA testing provides a useful conventional approach for preliminary bacterial identification. SSA can facilitate the isolation and differentiation of suspected enteric pathogens, while Gram staining provides information about bacterial morphology and Gram reaction. TSIA subsequently provides biochemical information that can strengthen the presumptive identification. Yue et al. [13], demonstrated that culture media may differ in their ability to recover and differentiate Salmonella, emphasizing the importance of appropriate media and laboratory procedures.

Nevertheless, the identification approach used in this study has limitations. The combination of colony morphology, Gram staining, and TSIA is not sufficient to definitively confirm Salmonella at the species or serovar level. Gram-negative bacilli and TSIA reactions similar to those observed in this study may also occur in other bacterial species. Molecular methods such as polymerase chain reaction (PCR) can provide more specific detection by targeting Salmonella-associated genetic markers [14][15]. Therefore, the findings of this study should be interpreted as presumptive identification and should ideally be confirmed using additional biochemical, serological, or molecular methods in future investigations.

The absence of bacterial growth in Sample D should likewise be interpreted within the limitations of the sampling and laboratory procedures. The result indicates that no bacterial colonies were detected under the examination conditions used in this study, but it does not necessarily demonstrate that the sample was completely free from Salmonella. Differences in bacterial concentration, sample distribution, dilution, culture recovery, and other analytical factors may influence the ability to detect microorganisms. Therefore, a negative culture result from a single sample should not be interpreted as absolute evidence of microbiological safety.

Overall, the findings demonstrate the value of combining laboratory examination with observations of food-handling environments. The laboratory results provide microbiological evidence, while field observations provide contextual information regarding conditions that may facilitate contamination. However, establishing specific sources and risk factors for Salmonella contamination would require a larger sample size, quantitative microbiological analysis, systematic assessment of food-handler practices, and confirmatory identification methods.

CONCLUSION

The laboratory examination demonstrated that five of the six meatball samples collected from selected snack areas in Gorontalo City showed bacterial characteristics consistent with the presumptive identification of *Salmonella* spp. The presumptive identification was supported by the combination of colony characteristics on Salmonella-Shigella Agar, Gram-negative rod-shaped bacterial morphology, and biochemical reactions on Triple Sugar Iron Agar, particularly the alkaline slant and acidic butt with hydrogen sulfide and gas production in most positive samples. In contrast, Sample D showed no bacterial colony growth under the examination conditions. These findings indicate that the examined meatballs may present a potential microbiological food safety concern, particularly considering the observed conditions of open food display, variable environmental cleanliness, and food-handling practices. However, the results cannot be considered definitive confirmation of *Salmonella* spp. because the identification procedures used in this study were presumptive and may produce similar characteristics in other bacteria. Therefore, future studies should involve a larger number of samples, systematic assessment of food-handler hygiene and environmental conditions, quantitative microbiological analysis, and confirmatory identification using additional biochemical, serological, or molecular methods such as PCR. Food vendors are also encouraged to improve personal hygiene, sanitation of utensils and food-contact surfaces, protection of meatballs from environmental exposure, and proper storage and handling practices to reduce potential foodborne contamination.

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