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General Microbiology Lab Report Staphylococcus Testing

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Abstract

Food contamination caused by pathogenic microorganisms remains a significant public health concern, particularly due to the presence of Staphylococcus aureus, a bacterium capable of producing toxins that may cause foodborne illnesses. This study aimed to determine the number of Staphylococcus bacterial colonies in food samples, evaluate their safety based on the maximum microbial contamination limits established by the Indonesian Food and Drug Authority (BPOM), and identify the presence of Staphylococcus aureus through catalase testing. The examination was conducted using chicken satay, chicken intestine satay, goat satay, meatballs, shredded chicken, and rendang samples. The samples were serially diluted using sterile water, inoculated onto Mannitol Salt Agar (MSA) medium using the pour plate method, incubated at 37°C for 24 hours, and subsequently analyzed through colony enumeration and catalase testing. The results showed variations in bacterial contamination levels among the tested samples. Chicken satay, chicken intestine satay, and goat satay exceeded the permitted microbial contamination limits, with colony counts of 135.2×10^4 CFU/mL, 49.4×10^4 CFU/mL, and 90×10^3 CFU/mL, respectively, and exhibited positive catalase reactions, indicating the presence of Staphylococcus spp. Meanwhile, meatballs, shredded chicken, and rendang met the safety requirements based on BPOM regulations. Positive catalase reactions were characterized by the formation of oxygen bubbles after exposure to hydrogen peroxide, confirming the presence of catalase-producing bacteria. These findings highlight the importance of proper sanitation, hygienic food handling practices, and microbial quality control to prevent foodborne diseases associated with Staphylococcus aureus contamination.

INTRODUCTION

Food refers to substances consumed by humans that provide energy and nutrients required for bodily functions and daily activities (Andriyani, 2019). It serves as a primary source of energy for metabolism and physical performance (Rismayanthi, 2015). However, improperly handled food and beverages may become contaminated with pathogenic microorganisms, leading to various foodborne diseases (Faridah & Silvia, 2019). Foodborne diseases are illnesses caused by the consumption of food contaminated with pathogenic microorganisms, including Staphylococcus aureus, one of the major bacterial pathogens associated with food poisoning (Bennett et al., 2013). Staphylococcus aureus is a Gram-positive coccus with a spherical morphology that commonly colonizes the nasal mucosa and skin of healthy individuals (Sato et al., 2019). This bacterium can produce toxins that cause food poisoning (Kadariya et al., 2014). Excessive microbial growth can alter organoleptic properties, reduce nutritional quality, and accelerate food deterioration (Aziz et al., 2020).

Pathogenic microorganisms present in food can pose health risks to consumers. Symptoms caused by the consumption of contaminated food may include nausea, vomiting, and abdominal cramps, with or without diarrhea (Rorong & Wilar, 2020). The catalase enzyme plays an important role in microbial survival by protecting cells against oxidative stress caused by hydrogen peroxide accumulation (Karimela et al., 2018). Therefore, catalase testing is commonly performed to detect catalase-producing bacteria, particularly aerobic and facultative anaerobic bacteria containing cytochrome systems (Services, 2015). The catalase test is also widely used to differentiate cocci bacteria, particularly between Staphylococcus and Streptococcus species (Hayati et al., 2019).

Staphylococcus aureus is widely recognized as one of the leading causes of bacterial food poisoning worldwide, particularly in foods that undergo extensive manual handling during preparation, such as street-vended and traditional foods (Kadariya et al., 2014). This bacterium produces several types of heat-stable enterotoxins that remain active even after cooking. Therefore, although bacterial cells may be destroyed through heating, previously produced toxins can remain in food and cause illness when consumed (Bennett et al., 2013). This characteristic makes S. aureus food poisoning difficult to prevent through cooking alone and highlights the importance of controlling bacterial growth throughout the food production chain, from raw material handling to preparation and storage (Rahayu & Nurwitri, 2019).

The detection of Staphylococcus in food samples is commonly performed using Mannitol Salt Agar (MSA), a selective and differential culture medium containing high concentrations of sodium chloride that inhibit the growth of most bacteria except salt-tolerant staphylococci (Services, 2015). MSA also contains mannitol and phenol red as an indicator. Bacteria capable of fermenting mannitol produce acid, resulting in a decrease in pH and a color change of the medium from red to yellow, whereas non-mannitol fermenters maintain the medium's original color (Karimela et al., 2018). Because Staphylococcus aureus is generally capable of fermenting mannitol, while many coagulase-negative staphylococci are not, colony characteristics on MSA can provide preliminary identification of S. aureus. However, further confirmation requires additional biochemical tests, such as catalase and coagulase tests (Hayati et al., 2019).

In Indonesia, microbial safety standards for processed and traditional foods are regulated by the Indonesian Food and Drug Authority (BPOM) through Regulation Number 13 of 2019, which establishes maximum microbial contamination limits for various food categories, including Staphylococcus contamination (BPOM RI, 2019). Traditional foods sold in open markets or by street vendors, such as satay, meatballs, and shredded meat products, are particularly vulnerable to microbial contamination due to handling practices, limited protective equipment, and prolonged exposure to room temperature before consumption (Sukmawati et al., 2018). Previous studies conducted in various regions of Indonesia have reported S. aureus contamination exceeding regulatory limits in traditional meat-based products, indicating that microbial contamination remains a recurring food safety concern (Rahayu et al., 2014; Putri et al., 2015).

The urgency of this research was driven by the increasing concern regarding foodborne illness cases in Indonesia, as reflected in recent BPOM findings related to the MBG program (Radar Kudus, 2026; Tribunbengkulu.com, 2026), as well as the widespread consumption of traditional meat-based foods with limited microbial safety monitoring. Understanding

contamination patterns and bacterial levels across different food types is essential for supporting targeted food safety interventions. The novelty of this study lies in the comparative analysis of six traditional meat-based food samples using standardized laboratory procedures to provide an overview of Staphylococcus contamination patterns across different preparation methods and food compositions. In addition, this study integrates microbiological education with food safety monitoring by providing practical experience in basic microbiological testing while generating preliminary data regarding local food safety conditions ((EFSA) et al., 2019; Datta et al., 2020; Makinde et al., 2020; Mota et al., 2021; Taiwo et al., 2024).

The catalase test is one of the simplest and most rapid preliminary methods used to differentiate catalase-positive bacteria, such as Staphylococcus, from catalase-negative bacteria, such as Streptococcus (Hayati et al., 2019). This test is based on the ability of catalase-producing bacteria to break down hydrogen peroxide (H_2O_2) into water and oxygen, which is indicated by visible bubble formation after exposure to hydrogen peroxide (Karimela et al., 2018). Since Staphylococcus aureus produces catalase, the test is frequently combined with colony enumeration on MSA as a screening method in food microbiology, particularly in educational and field laboratory settings where advanced molecular identification methods, such as polymerase chain reaction (PCR), may not always be available (Aziz et al., 2020).

Globally, foodborne illnesses caused by pathogenic bacteria such as Staphylococcus aureus continue to pose significant public health challenges, with outbreaks frequently associated with foods requiring extensive manual handling before consumption, including cooked meat products, sauces, and ready-to-eat foods (Bennett et al., 2013). In developing countries, including Indonesia, the widespread consumption of traditional foods prepared by small-scale vendors highlights the importance of continuous microbial safety monitoring because these products are consumed by large populations while often having limited formal quality control systems (Faridah & Silvia, 2019). Laboratory activities involving microbial identification therefore serve not only as practical training in microbiological techniques but also as a means of generating preliminary information regarding local food safety conditions.

Based on this background, this study aimed to: (1) determine the number of Staphylococcus bacterial colonies in the tested food samples; (2) evaluate the safety status of the samples based on BPOM microbial contamination limits; and (3) identify the presence of Staphylococcus aureus through catalase testing.

METHOD

The Staphylococcus examination was conducted in Group III using chicken intestine satay samples. Before the practicum, the laboratory table was sterilized using Wipol, and the sample preparation process was performed by diluting the sample in sterile water. The chicken intestine satay sample was crushed into small pieces, weighed as much as 5 g, and transferred into a bottle containing 45 mL of sterile water for dilution. The bottle containing the sample suspension was then homogenized by shaking until the solution became cloudy. A Bunsen burner was ignited, and a Petri dish was prepared near the flame to minimize contamination. A 1 mL aliquot of the diluted sample was transferred into a Petri dish using a micropipette. The sample was then mixed with Mannitol Salt Agar (MSA) medium using the pour plate method. The Petri dish was kept near the Bunsen burner during the inoculation process to reduce microbial contamination. The edges of the Petri dish were sealed with plastic wrap and

incubated at 37°C for 24 hours. After incubation, the number of bacterial colonies was counted, followed by a catalase test using hydrogen peroxide. The formation of oxygen (O_2) bubbles indicated the presence of catalase-producing bacteria, including Staphylococcus spp.

RESULTS AND DISCUSSION

The results of observations in group I with chicken satay samples were obtained as a result of the number of colonies that did not meet the criteria, namely 135.2 x 104 CFU/mL with positive catalase test results. The result of the positive catalase test was marked by the presence of O2 bubbles produced after being dripped by hydrogen peroxide (H2O2) on the glass of the object, this indicates that there is *Staphylococcus sp bacteria* in the sample. The results of observation in group II with meatball samples were obtained as a result of the number of colonies that met, namely with 0 (not growing) colonies in the media, and the results of the catalase test were negative. The result of a positive catalase test is characterized by the presence of O2 bubbles produced after being dripped by hydrogen peroxide (H2O2) on the glass of the object. This indicates that there is *Staphylococcus sp bacteria* in the sample.

The results of observations in group III with chicken intestine satay samples were obtained as a result of the number of colonies that did not meet the criteria, namely 49.4 x 104 CFU/mL with positive catalase test results. The result of a positive catalase test is characterized by the presence of O2 bubbles produced after being dripped by hydrogen peroxide (H2O2) on the glass of the object. This indicates that there is *Staphylococcus sp bacteria* in the sample. The results of observations in group IV with goat satay samples were obtained as a result of the number of colonies that did not meet the number of colonies, namely 90 x 103 CFU/mL with positive catalase test results. The result of a positive catalase test is characterized by the presence of O2 bubbles produced after being deposited by hydrogen peroxide (H2O2) on the glass of the object. This indicates that there is *Staphylococcus sp bacteria* in the sample. The results of observation in group V with shredded chicken samples were obtained as a result of the number of colonies that met, namely with 0 (not growing) colonies and negative catalase test results. The results of observation in group VI with rendang samples were obtained as a result of the number of colonies that met, namely 200 x 101 CFU/mL with positive catalase test results. The result of a positive catalase test is characterized by the presence of O2 bubbles produced after being dripped by hydrogen peroxide (H2O2) on the glass of the object. This indicates that there is *Staphylococcus sp bacteria* in the sample.

Group I with chicken satay samples showed results that were not suitable for consumption because they were less than the bacterial contamination limit, which was 102 CFU/mL listed in the regulations of the Food and Drug Supervisory Agency of the Republic of Indonesia in 2019. The cause of contamination in chicken satay is due to contamination from the soil during meat cutting, dirt on the skin, contents of the digestive tract, water, and tools used during the preparation process (Rahayu et al., 2014). Group II with meatball samples showed the results that meatballs were suitable for consumption because they did not grow colonies in *the Manitol Salt Agar* (MSA) media. The feasibility of consumption from food is influenced by sanitation, hygienic factors and proper handling during processing (Sukmawati et al., 2018). Group III with chicken intestine satay samples showed results that were not suitable for consumption because they were less than the bacterial contamination limit of 102 CFU/mL listed in the regulations of the Food and Drug Control Agency of the Republic of

Indonesia in 2019. This can be caused by the presence of preservatives in chicken intestinal satay samples such as formalin which can kill *S. aureus bacteria* (Irvanda et al., 2018). Or the number of colonies that exceed the maximum limit can be caused by contamination in the production and processing process of unhygienic foodstuffs (Kadariya *et al*., 2014).

Group IV with goat satay samples showed results that were not suitable for consumption because they were less than the bacterial contamination limit, which was 102 CFU/mL listed in the regulation of the Food and Drug Supervisory Agency of the Republic of Indonesia in 2019. The factors that cause the presence of *S. aureus* are due to the improper meat washing process so that *S. aureus* can still grow (Aisyah, 2019). Group V with shredded chicken samples showed results that shredded chicken was suitable for consumption because it lacked colony growth on *Manitol Salt Agar* (MSA) media. Feasibility of consumption because it does not exceed the limit of bacterial contamination listed in the 2019 BPOM RI regulation. Samples that meet hygienic and sanitary requirements both have safety and feasibility of the processing process (Maulana *and* Nandiyanto, 2022). Group VI with rendang samples is a sample suitable for consumption because it does not exceed the maximum limit of S. *aureus* in processed meat products in BPOM. Processed foods are still suitable for use even though *S. aureus is present*. This can occur due to contamination samples during product preparation or the presence of feces on animals (Putri et al., 2015). In addition, the factors that cause the presence of *S. aureus* are due to the pH and humidity of the liver meat that are suitable for overgrowth (Rahayu and Nurwitri, 2019).

Table 1. Summary of colony counts, catalase test results, and consumption feasibility of the six food samples based on BPOM RI (2019) microbial contamination limits

Group	Sample	Colony Count (CFU/mL)	Catalase Test	BPOM Status
I	Chicken satay	135.2×10^4	Positive	Not suitable
II	Meatballs	0 (no growth)	Negative	Suitable
III	Chicken intestine satay	49.4×10^4	Positive	Not suitable
IV	Goat satay	90×10^3	Positive	Not suitable
V	Shredded chicken	0 (no growth)	Negative	Suitable
VI	Rendang	200×10^1	Positive	Suitable

As summarized in Table 1, three of the six samples examined, namely chicken satay, chicken intestine satay, and goat satay, exceeded the maximum permitted microbial contamination limit and were therefore classified as unsuitable for consumption, while the remaining three samples, meatballs, shredded chicken, and rendang, met the safety requirements set out in the BPOM regulation. This pattern suggests that satay-type products, which involve extensive manual handling such as cutting, skewering, and grilling over an open flame, may be more prone to microbial contamination than products that undergo more standardized processing, boiling, or the addition of preservative spices, as is typically the case in the preparation of rendang (Rahayu et al., 2014).

Several factors are believed to contribute to the elevated *Staphylococcus* contamination observed in the non-compliant samples. First, *S. aureus* is part of the normal microbiota of human skin and the nasal cavity, so food handlers who do not practice adequate personal hygiene, such as washing hands thoroughly before handling food or wearing gloves and masks, can easily transfer the bacteria onto food during preparation (Sato et al., 2019). Second, traditional foods such as satay are commonly prepared and sold in open-air settings using

simple equipment that is difficult to sanitize completely, which increases the opportunity for cross-contamination from cutting boards, knives, and serving utensils (Rahayu et al., 2014). Third, temperature abuse is likely to play a role, as satay and similar products are often displayed and sold at ambient temperature for extended periods, providing favorable conditions for *Staphylococcus* to multiply and, if present in sufficient numbers, to produce enterotoxins (Kadariya et al., 2014). In contrast, the lower contamination observed in meatballs and shredded chicken may be attributed to their preparation process, which typically involves boiling or steaming at temperatures sufficient to reduce the bacterial load, together with the relatively enclosed handling process used in their production (Sukmawati et al., 2018).

It is important to note that colony counting on MSA and the catalase test used in this practicum provide only a preliminary indication of *Staphylococcus* contamination and do not conclusively confirm the presence of *Staphylococcus aureus* specifically. Several coagulase-negative *Staphylococcus* species are also catalase-positive and capable of growing on MSA, so a positive catalase result combined with yellow colony formation is only a strong presumptive indicator rather than a definitive identification (Hayati et al., 2019). Confirmatory tests, such as the coagulase test or molecular identification using Polymerase Chain Reaction (PCR), would be required to establish the presence of *S. aureus* with greater certainty (Aziz et al., 2020). In addition, because only a single sample was examined for each food type in this practicum, the results should be interpreted as an indicative snapshot rather than a comprehensive assessment of the microbial safety of each food category as a whole.

Based on these findings, several practical measures can be recommended to reduce the risk of *Staphylococcus aureus* contamination in traditionally prepared foods. Food handlers, particularly those preparing satay and similar ready-to-eat products, should be trained in basic food hygiene practices, including handwashing, the use of gloves, and the proper cleaning of utensils and cutting surfaces, in line with the sanitation principles emphasized by Sukmawati et al. (2018). Vendors are also encouraged to minimize the length of time that cooked products are displayed at ambient temperature and, where possible, to use insulated or temperature-controlled storage to slow bacterial growth. From a regulatory perspective, periodic microbial monitoring of traditional foods sold in markets, consistent with the standards established by BPOM RI (2019), would help identify unsafe products before they reach consumers and support targeted interventions in food safety education for vendors.

The findings of this practicum are broadly consistent with the principles underlying Hazard Analysis and Critical Control Point (HACCP) approaches to food safety, which emphasize identifying the specific stages of food handling most likely to introduce or allow the growth of pathogenic bacteria (Rahayu and Nurwitri, 2019). In the case of satay-type products, the cutting and skewering stages, along with prolonged holding at ambient temperature after grilling, can reasonably be identified as critical points at which contamination is introduced or *Staphylococcus* is allowed to multiply. Addressing these specific points through improved handling practices and shorter holding times, rather than relying on cooking temperature alone, is therefore likely to be more effective in reducing contamination than general hygiene advice. This practicum illustrates, on a small scale, how routine microbiological screening using accessible methods such as MSA culturing and the catalase test can support this kind of targeted food safety assessment even in settings without access to more sophisticated laboratory infrastructure.

CONCLUSION

This study demonstrates that Staphylococcus aureus contamination remains a significant public health concern in traditional meat-based foods in Indonesia. The presence of Staphylococcus aureus was indicated by yellow colony formation on Mannitol Salt Agar (MSA) medium and bubble formation during the catalase test. The results showed that meatballs, shredded chicken, and rendang met the safety requirements based on BPOM Regulation Number 13 of 2019, whereas chicken satay, chicken intestine satay, and goat satay exceeded the permitted Staphylococcus contamination limits and were considered unsafe based on the applicable standards. The positive catalase reactions observed in the non-compliant samples confirmed the presence of catalase-producing bacteria, including Staphylococcus spp. These findings highlight the importance of proper sanitation, hygienic food handling practices, and microbial quality control to reduce the risk of foodborne diseases associated with Staphylococcus aureus contamination.

This study had several limitations. Only one sample was examined for each food type, and the identification process was limited to basic microbiological methods using MSA culture and catalase testing without further confirmation through coagulase testing or molecular identification techniques. In addition, the study did not investigate the specific sources of contamination or the enterotoxin-producing potential of the isolated bacteria. Future studies should include larger sample sizes, multiple sampling locations, and different sampling periods to provide a more comprehensive assessment of Staphylococcus contamination patterns across regions and seasons. Confirmatory methods, such as coagulase testing, polymerase chain reaction (PCR), or sequencing, should be applied to verify the presence of Staphylococcus aureus and identify potential enterotoxin-encoding genes. Further investigations into antimicrobial resistance profiles of isolated S. aureus strains and the effectiveness of hygiene interventions in reducing contamination levels are also recommended. Moreover, studies examining the relationship between food handler hygiene practices and contamination levels could provide valuable evidence for developing targeted food safety education programs for street food vendors and traditional food handlers.

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