

Identification of Bacteria on Food Utensils at Food Stalls along H.A.M.M. Rifaddin Street, Samarinda City

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ABSTRACT

Food utensils can serve as a medium for bacterial contamination when hygiene and sanitation practices are inadequate. This study aimed to describe the hygiene and sanitation conditions and identify bacteria recovered from food utensils at food stalls along H.A.M.M. Rifaddin Street, Samarinda City. This descriptive observational study involved 12 food stalls and 48 utensil swab samples consisting of plates, glasses, spoons, and forks. Bacterial identification was performed using culture, Gram staining, and biochemical tests, while hygiene and sanitation conditions were assessed using an observation checklist. The results showed that 11 food stalls (91.7%) had good hygiene and sanitation conditions and 1 stall (8.3%) had poor conditions. Bacterial growth was detected in all 48 samples. All isolates were Gram-negative bacilli and were identified as *Citrobacter* sp. (45.8%), *Citrobacter freundii* (31.3%), and *Enterobacter* sp. (22.9%). These findings indicate that bacterial growth was detected on all examined utensils despite generally favorable observed hygiene and sanitation conditions. Because quantitative microbial counts and specific numerical microbiological parameters were not examined, the findings cannot be used to definitively determine compliance with numerical microbiological standards.

INTRODUCTION

Foodborne disease remains an important public health problem. Microbial contamination can occur throughout food handling, including preparation, serving, and contact with utensils that do not meet hygiene and sanitation requirements. Good hygiene and sanitation practices are therefore important components of food safety (WHO, 2022).

Food utensils come into direct contact with food and may serve as vehicles for the transfer of microorganisms. Utensils that appear physically clean are not necessarily microbiologically clean. Contamination may occur because of ineffective washing, unsuitable washing water, unhygienic wiping cloths, inadequate drying, or open storage, which may facilitate cross-contamination (CAC, 2022; FAO & WHO, 2022).

Indonesian Ministry of Health Regulation Number 2 of 2023 provides environmental health requirements for food-handling establishments, including requirements concerning the cleanliness, drying, and protection of food-contact utensils. Microbiological examination can provide additional information because visual observation alone cannot demonstrate the presence or absence of microorganisms.

Previous studies have reported bacterial contamination on food utensils. Pradina (2018) reported bacterial contamination on utensils used by meatball vendors, while Fadhillah et al. (2023) reported bacterial contamination on utensils used by hospital patients and contamination of washing water. At the same 12 food stalls examined in the present study, Rezky (2025) previously reported microbial counts exceeding the permitted level, with the highest mean count on forks (32.2 CFU/cm²) and the lowest on spoons (4.0 CFU/cm²).

The novelty of the present study lies in its extension of the previous quantitative study by Rezky (2025) at the same 12 food stalls along H.A.M.M. Rifaddin Street, Samarinda City. While the previous study focused on quantifying bacterial contamination on food utensils, the present study provides presumptive bacterial identification through culture, Gram staining, and biochemical testing. It also evaluates the observed hygiene and sanitation practices of the food stalls and describes these findings alongside the microbiological results. By combining bacterial identification with hygiene and sanitation observations in the same study setting, this research provides complementary information to the previously reported quantitative data.

The present study was conducted to provide further information by identifying bacteria recovered from food utensils and describing the hygiene and sanitation conditions of the food stalls. The study also examined the microbiological findings in relation to the requirements of Ministry of Health Regulation Number 2 of 2023, while recognizing the limitations of culture-based identification without quantitative microbial testing.

LITERATURE REVIEW

Food Utensils and Hygiene and Sanitation Requirements

Food utensils are items used in the serving, temporary storage, and consumption of food or beverages that come into direct contact with food, including plates, glasses, spoons, forks, bowls, and other items used during food service. Utensil cleanliness is an important aspect of food safety because utensils can transfer microorganisms to food if they are not properly cleaned and sanitized (Codex Alimentarius Commission [CAC], 2022; WHO, 2022). Under Ministry of Health Regulation Number 2 of 2023, food utensils must be free from food residue, oil, dust, rust, odor, and pathogenic microorganisms. The washing process should include removal of food residue, washing with detergent, rinsing with water that meets quality requirements, air drying, and storage in a closed space to prevent recontamination (Kementerian Kesehatan RI, 2023).

Bacterial Contamination of Food Utensils

Bacteria are widely distributed in the environment and may be associated with food, water, soil, animals, and humans. Several members of the order Enterobacterales are facultatively anaerobic Gram-negative bacilli, including genera such as *Escherichia*, *Salmonella*, *Citrobacter*, and *Enterobacter*. Some species within these genera are recognized as enteric or opportunistic pathogens and may be associated with food contamination (Bria et al., 2022).

Factors Associated with Bacterial Contamination

Several interrelated factors may influence bacterial contamination of food utensils. The quality of washing water is important because water that does not meet microbiological standards may recontaminate utensils even after detergent washing (WHO, 2022). The washing process also determines how effectively food residue, fat, and protein are removed from utensil surfaces (CAC, 2022). Rinsing with running water can assist in removing residual detergent and microorganisms compared with reuse of standing water (Centers for Disease Control and Prevention [CDC], 2024).

Improper drying, particularly the repeated use of wiping cloths, may leave utensils damp and provide an opportunity for microbial persistence, whereas air drying on a clean rack is recommended (Food and Agriculture Organization [FAO] & WHO, 2022). Open or crowded storage of damp utensils, together with inadequate food-handler hand hygiene, may increase the possibility of recontamination after washing (CDC, 2024; Kementerian Kesehatan RI, 2023).

Conceptual Framework

Because this study is descriptive rather than hypothesis-testing, no statistical hypotheses were formulated. The study was guided by a conceptual framework linking hygiene and sanitation practices during utensil handling—including washing, rinsing, drying, and storage—to bacterial growth on food utensils. Hygiene and sanitation conditions were assessed through direct observation, while bacterial growth and

identification were assessed through culture, Gram staining, and biochemical testing. The findings from the two assessments were then compared descriptively to describe the pattern between observed hygiene and sanitation practices and the microbiological findings.

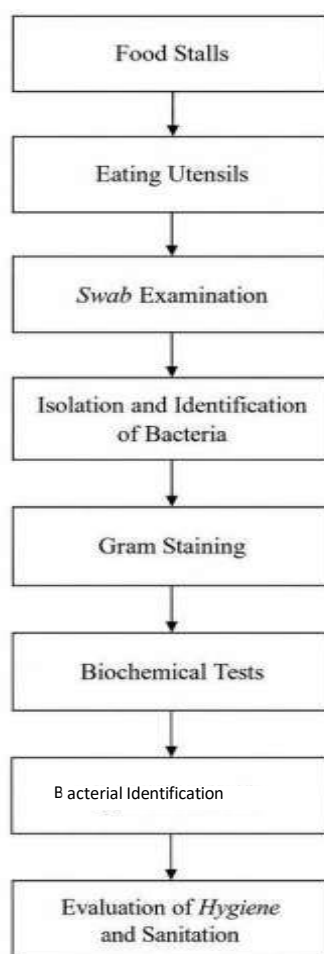


Figure 1. Conceptual Framework

METHODOLOGY

This study used a descriptive observational design with a cross-sectional approach. The study was conducted at food stalls along H.A.M.M. Rifaddin Street, Samarinda City, with laboratory examinations performed at the Bacteriology Laboratory of the Department of Medical Laboratory Technology, Poltekkes Kemenkes Kalimantan Timur. The study was conducted on 8 December 2025.

The study population comprised all food stalls along H.A.M.M. Rifaddin Street that met the inclusion criteria: being located along the street, operating during the study period, using plates, glasses, spoons, and forks, and having owners or managers who agreed to observation and sampling. A total of 12 stalls met these criteria, and all were included using a total sampling technique. Therefore, no separate sample-size calculation was performed, as the study aimed to describe bacterial identification findings in the entire eligible population rather than estimate population parameters or

test hypotheses. The same 12 stalls had previously been examined quantitatively by Rezky (2025), allowing the present bacterial identification findings to be discussed in relation to the previously reported bacterial counts. One plate, one glass, one spoon, and one fork were collected from each stall, yielding 48 utensil swab samples. The findings are limited to the sampled stalls and should not be generalized to other stalls or areas without further investigation.

The main variable was bacterial identification on food utensils, supported by observational data on the hygiene and sanitation conditions of the food stalls and food utensils, referring to Ministry of Health Regulation Number 2 of 2023. Hygiene and sanitation conditions of the stalls were categorized as good or poor, utensil hygiene and sanitation as compliant or non-compliant, bacterial growth as positive or negative, and utensil microbiological status as meeting or not meeting the applicable requirements.

The hygiene and sanitation assessment covered the checklist items related to utensil handling, including washing practices, use of dishwashing detergent, water sanitation, drying, and utensil storage. Laboratory examination assessed bacterial growth, Gram-staining characteristics, and presumptive bacterial identification based on biochemical reactions. However, the methods did not include quantitative microbial counts or specific pathogen testing to determine compliance with numerical microbiological limits. Therefore, the assessment was limited to the observed hygiene and sanitation practices and the microbiological findings obtainable through the methods employed.

Utensil surfaces that directly contacted food were swabbed using sterile swabs pre-moistened with Brain Heart Infusion (BHI) broth. Swabbing was performed horizontally, vertically, and diagonally. Samples were placed in labeled sterile tubes, transported in a cool box, and examined in the laboratory.

Isolation was performed on Blood Agar and MacConkey Agar using aseptic technique, followed by incubation at 35–37°C for 18–24 hours. Colony morphology was observed, and pure colonies were subjected to Gram staining. Biochemical characterization was performed using Triple Sugar Iron Agar (TSIA), Sulfur Indole Motility (SIM), Simmons citrate (SC), Methyl Red (MR), and Voges-Proskauer (VP) tests. Presumptive identification was based on the combined biochemical reaction patterns and microbiological references. Hygiene and sanitation conditions were assessed using an observation checklist. Data were processed through editing, coding, and tabulation and were presented as frequency distributions and percentages. Hygiene and sanitation observations were compared descriptively with the microbiological findings; no inferential statistical test was performed.

RESEARCH RESULTS

This study involved 12 food stalls along H.A.M.M. Rifaddin Street, Samarinda City. Hygiene and sanitation conditions were assessed using an observation checklist based on Ministry of Health Regulation Number 2 of 2023. Swab samples were collected from four types of food utensils – plates, glasses, spoons, and forks—yielding 48 samples examined through culture, Gram staining, and biochemical testing. At each stall, hygiene sanitation was assessed using a checklist based on Ministry of Health Regulation Number 2 of 2023, and swab samples were collected from four types of eating utensils (plates, glasses, spoons, and forks), yielding 48 samples examined microbiologically through culture, Gram staining, and biochemical testing.

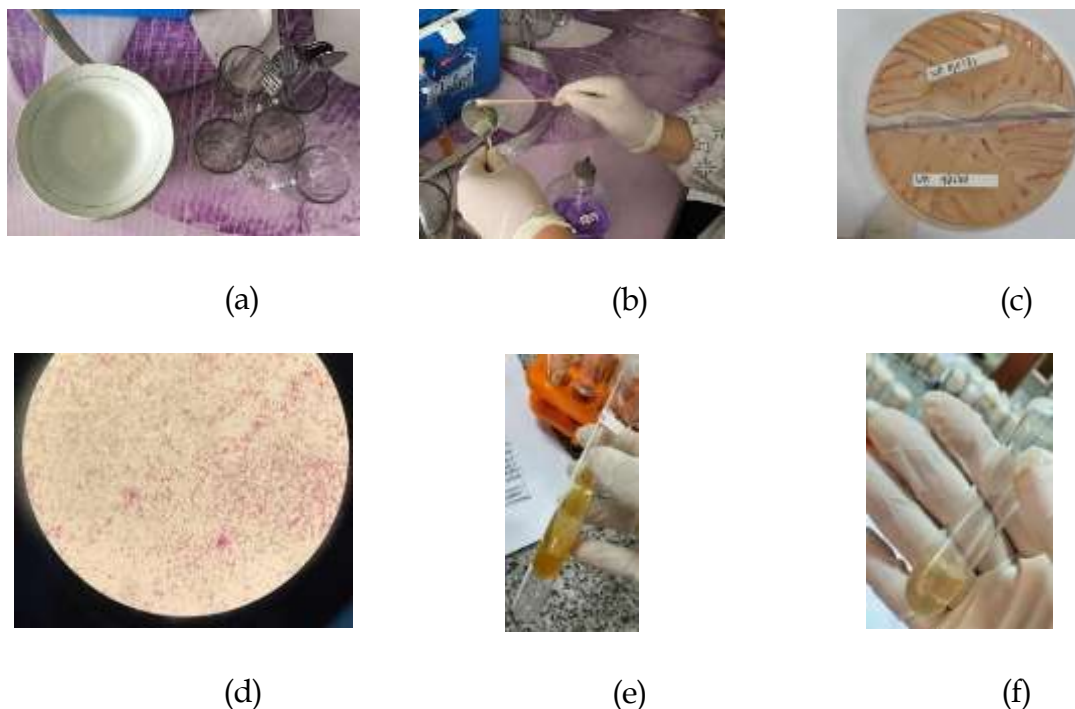


Figure 2. Documentation of bacterial isolation and identification procedures: (a) utensil preparation; (b) swab collection; (c) bacterial culture; (d) Gram staining; (e) TSIA test; (f) SIM test; (g) MR test; (h) VP test; and (i) citrate test.

Hygiene Sanitation Condition of Food Stalls

Table 1. Hygiene and Sanitation Conditions of Food Stalls along H.A.M.M. Rifaddin Street, Samarinda City

Hygiene Sanitation Category	Number of Stalls	Percentage (%)
Good	11	91.7
Poor	1	8.3
Total	12	100

Most food stalls had implemented most components of hygiene sanitation, including provision of clean water, dedicated washing areas, waste disposal facilities, and use of washing detergent. However, several practices remained suboptimal, such as open-rack storage of utensils,

repeated use of wiping cloths, and inconsistent air drying, all of which could allow recontamination after washing.

Hygiene Sanitation Condition of Eating Utensils

Table 2. Hygiene Sanitation Aspects of Eating Utensils at Food Stalls on H.A.M.M. Rifaddin Street, Samarinda City

Hygiene Sanitation Aspect	Good (n)	Poor (n)
Water sanitation	10	2
Use of dishwashing soap	12	0
Specific washing method	0	12
Utensil storage	12	0

All 12 stalls used physically intact utensils and washed them with detergent and running water, and stored washed utensils in a clean place. Nevertheless, 8 stalls (66.7%) did not use hot water for sanitizing utensils, 4 stalls (33.3%) still dried utensils with wiping cloths, and 3 stalls (25%) stored utensils in uncovered containers, indicating that post-washing handling remained a potential source of recontamination.

Culture Examination

Table 3. Bacterial Growth on Food Utensils Based on Culture Examination

Culture Result	Number of Samples	Percentage (%)
Bacterial growth detected	48	100
No bacterial growth detected	0	0
Total	48	100

Bacterial growth was detected in all 48 utensil samples. This finding indicates that viable bacteria were recovered from all examined food-contact surfaces.

Gram Staining

Table 4. Bacterial Characteristics Based on Gram Staining

Gram Stain Result	Number of Isolates (n)	Percentage (%)
Gram-positive	0	0
Gram-negative	48	100
Total	48	100

All recovered isolates were Gram-negative bacilli. No Gram-positive isolates or coccal forms were detected. Further biochemical testing was therefore performed for presumptive genus- or species-level identification.

Biochemical Characterization

Table 5. Bacterial Characteristics Based on Biochemical Examination

Bacterial Species	TSIA	SIM (H ₂ S/Indole/ Motility)	SC	MR	VP	Isolates (n)
<i>Citrobacter</i> sp.	K/K, H ₂ S(-), Gas(+)	(-) / (-/+) / (+)	+	+	-	22
<i>Citrobacter freundii</i>	K/K, H ₂ S(-), Gas(+)	(+) / (-) / (+)	+	+	-	15
<i>Enterobacter</i> sp.	K/K, H ₂ S(-), Gas(+)	(-) / (-) / (+)	+	-	+	11

Based on the combined culture morphology, Gram-stain characteristics, and biochemical reaction patterns, 22 isolates were presumptively identified as *Citrobacter* sp. (45.8%), 15 isolates as *Citrobacter freundii* (31.3%), and 11 isolates as *Enterobacter* sp. (22.9%). All identified isolates belonged to the order Enterobacterales.

DISCUSSION

The observation showed that most food stalls were categorized as having good hygiene and sanitation conditions. Nevertheless, several aspects of utensil handling still required attention, particularly the washing method and practices occurring after washing. Hygiene and sanitation assessment is intended to control contamination risks arising from food, people, places, and equipment; therefore, assessment of the entire handling sequence remains important (CAC, 2022; FAO & WHO, 2022).

Bacterial growth was detected in all 48 samples, indicating that viable bacteria were recovered from the examined food-contact surfaces after routine handling. Bacterial presence may be influenced by washing effectiveness, water quality, rinsing, drying, storage, and cross-contamination during post-washing handling (CAC, 2022; Gurtler et al., 2023). However, because the present study did not investigate specific contamination sources or risk factors, the observed practices cannot be stated to be direct causes of bacterial growth. The detection of bacterial growth does not necessarily indicate that all examined utensils pose an immediate food-safety risk. The actual risk depends on the bacterial species, its pathogenic potential, and the level of contamination. In this study, the isolates were presumptively identified as *Citrobacter* sp., *Citrobacter freundii*, and *Enterobacter* sp. However, quantitative microbial counts and further confirmation of pathogenic organisms were not performed. Therefore, the findings demonstrate the presence of bacteria on the examined utensils but are insufficient to determine the actual level of food-safety risk.

All recovered isolates were Gram-negative bacilli. Gram staining is an initial identification step and cannot by itself establish bacterial species. Gram-negative bacilli with similar morphology may have different metabolic profiles; therefore, Gram-staining results should be interpreted together with culture characteristics and biochemical reactions (Forbes et al., 2023; Koneman et al., 2017).

The biochemical profiles allowed presumptive identification of the isolates as *Citrobacter* sp., *Citrobacter freundii*, and *Enterobacter* sp. The differentiation was based on the combined reaction patterns of TSIA, SIM, Simmons citrate, MR, and VP rather than on a single test. Conventional biochemical identification depends on the interpretation of reaction patterns; therefore, more specific confirmation using automated identification or molecular methods could improve taxonomic resolution in future studies.

The discrepancy between the generally favorable observational hygiene and sanitation assessment and the detection of bacterial growth in all examined samples indicates that these two approaches provide complementary information. Observation describes hygiene and sanitation practices and environmental conditions at the food stalls, whereas laboratory examination provides information on the microbiological status of the sampled utensil surfaces. Because this study was descriptive and did not investigate causal relationships, the findings should not be interpreted as evidence that a particular sanitation practice directly caused bacterial growth.

With respect to Ministry of Health Regulation Number 2 of 2023, this study assessed selected hygiene and sanitation practices through an observation checklist and examined bacterial growth and presumptive bacterial identity using culture, Gram staining, and biochemical tests. These methods provided information on observed handling practices and the presence and characteristics of recovered bacteria. However, quantitative microbial counts and specific tests to confirm relevant pathogenic organisms were not performed. Consequently, compliance with numerical microbiological limits and the presence or absence of specific pathogens could not be conclusively established. The findings should therefore be interpreted within the scope of the parameters assessed rather than as a comprehensive determination of compliance with all applicable requirements.

CONCLUSIONS AND RECOMMENDATIONS

Observation showed that 11 of 12 food stalls (91.7%) had good hygiene and sanitation conditions, while one stall (8.3%) had poor conditions. Bacterial growth was detected in all 48 utensil samples, and all recovered isolates were Gram-negative bacilli. Based on biochemical testing, 22 isolates (45.8%) were presumptively identified as *Citrobacter* sp., 15 isolates (31.3%) as *Citrobacter freundii*, and 11 isolates (22.9%) as *Enterobacter* sp. These findings indicate that observational assessment of hygiene and sanitation did not fully reflect the microbiological findings obtained from the examined utensils. Because quantitative microbial counts and specific numerical microbiological parameters were not examined, the findings should be interpreted as evidence of bacterial presence rather than as a definitive determination of compliance with numerical microbiological standards.

Food-stall operators are advised to strengthen hygiene and sanitation practices throughout washing, rinsing, drying, and storage; avoid repeated use of wiping cloths; and store washed utensils in clean and protected

conditions. Health authorities may complement routine hygiene and sanitation inspections with periodic microbiological examinations. Future studies should examine potential contamination sources, including washing water, wiping cloths, storage racks, and food handlers' hands, and may use quantitative methods and automated or molecular identification techniques.

ADVANCED RESEARCH

This study was limited to 12 food stalls along H.A.M.M. Rifaddin Street, Samarinda City; therefore, the findings describe only the sampled stalls and cannot be generalized to other locations without further investigation. Bacterial identification relied on conventional culture, Gram staining, and biochemical testing, which provided presumptive identification and may have lower taxonomic resolution than automated or molecular methods. In addition, the study did not include quantitative microbial counts, confirmatory testing for specific pathogenic organisms, or investigation of potential contamination sources. Consequently, the findings cannot establish the overall prevalence of contamination or determine the actual level of food-safety risk. Future studies should include a wider geographic area and more food stalls, quantitative microbial testing, confirmation of bacterial identity using automated or molecular methods, and investigation of potential contamination sources.

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