

Bacterial isolates from chapati sold at Shilabela market, Geita district, Tanzania. A cross-sectional study.

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Abstract

Background

Chapatis are a traditional Indian flatbread made with whole wheat flour. The study aims to isolate and identify bacterial pathogens from chapatti collected from Shilabela Market, Geita District, Tanzania.

Methodology

A laboratory-based cross-sectional study was conducted at Chapatti market stalls in Shilabela Market from May to June 2025. The primary study population included chapati samples sold by vendors at Shilabela market, and the secondary study population included the chapati vendors operating in Shilabela market. Data were summarized into tables, bar graphs, and pie charts using Excel.

Results

Of the 112 chapatti samples cultured, 65 samples (58.0%) exhibited growth on MacConkey Agar, indicating the presence of Gram-negative enteric bacteria. Mannitol Salt Agar showed positive growth in 55 samples (49.1%), suggesting *Staphylococcus* species. Positive hydrolysis was exhibited in 32 samples (28.6%) on Bile Esculin Agar, which suggested the possible presence of *Enterococcus* or Group D *Streptococci*. 87 isolates (57.2%) were Gram-positive, largely from subcultures extracted from the Mannitol Salt Agar and the Bile Esculin Agar. The remaining 65 isolates (42.8%) were Gram-negative and were entirely drawn from subcultures utilising MacConkey Agar. This pattern followed the unique selective ability of the respective cultural media. *Staphylococcus aureus* was the most common isolate at 32.9%, and *E. coli* was second at 29.6%, followed by *Enterococcus* spp. (19.7%) and *Klebsiella* spp. (9.9%).

Conclusion

Bacterial pathogens have been isolated and identified from chapatti sold at Shilabela Market, Geita District, with a contamination profile dominated by *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus* spp., and *Klebsiella* spp.

Recommendations

Food vendors operating in the Shilabela Market should participate in regular and thorough training programs related to food safety that improve their knowledge of hygiene practices and reduce the potential risk of bacterial contamination in chapatti products.

Keywords: Bacterial pathogens, Chapatti, Shilabela Market, Geita District

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Background

Chapatis are a traditional Indian flatbread made with whole wheat flour. The origins of the popularity of

chapatis in Tanzania are unclear, but it may have something to do with the Indian immigrants who have been in the country since the colonial era, at the turn of the 19th century (*Popular Street Food in Uganda | D+C - Development + Cooperation*, 2024). There are many

advantages that are offered by the street-vended foods, including chapatti, but evidence does exist that the foods that are exposed for sale on the roadside may potentially be contaminated by many pathogenic microorganisms, including different bacterial species. However, very clear information on bacteriological profiles, bacterial load, as well as the antimicrobial susceptibility patterns of the bacterial isolates from these street foods, like chapatti, is lacking (Amare et al., 2019).

The isolation and identification of bacterial pathogens from chapatti is important in food safety and public health issues. Chapatti is a staple food that can easily be contaminated during handling and storage. If food-borne illnesses related to bacterial pathogens lead to severe consequences, identifying pathogens in chapatti is an important step in understanding the risks associated with chapatti and finding ways to control these risks (Elbehiry et al., 2023).

There is an accumulating global evidence generally indicating that ready-to-eat (RTE) foods are often heavily contaminated with pathogens. Broader, macro-level studies from Asia and South America routinely identify *E. coli*, *Salmonella spp.*, *Klebsiella spp.*, and *S. aureus* from RTE foods, all of which were isolated using standard microbiological protocols and media (MacConkey Agar; Mannitol Salt Agar; and biochemical confirmation tests). This includes proper ways of isolating pathogenic bacteria from the chapatti in this research (Fusaro et al., 2024).

In Africa, studies routinely find the same pathogens in street food. In Ethiopia, studies found *E. coli*, *S. aureus*, *Salmonella*, and *Klebsiella* in RTE foods using selective and differential media. These observational studies demonstrate that the methods used for isolation of pathogens are still useful and a legitimate part of food safety surveillance (Moges et al., 2025). At the same time, in Nigeria, 120 RTE food samples (fufu, zobo, etc.) were subjected to 16S rRNA sequencing to isolate *Enterobacter hormaechei*, *Klebsiella*, and *Staphylococcus*, some of which were verified with PCR methods (Makinde et al., 2021). These data also confirm the reliability of selective media and confirmatory tests for the detection of other pathogens in chapatti.

A study in Nairobi, Kenya, stated that several governments in Africa have shown interest in blending wheat flour, both to mitigate food security risks and to draw demand for traditionally grown but underutilised crops (Kariuki et al., 2025). The study aims to isolate and identify bacterial pathogens from chapatti collected from Shilabela Market, Geita District, Tanzania.

Materials and methods

Study design

This is a laboratory-based cross-sectional study that was carried out in Chapatti market stalls of Shilabela Market from May to June 2025. Chapatti slices were collected aseptically and conveyed to Sakamu Hospital Microbiology laboratory for microbiological analysis using standard analytical methods. This study design is cheap and easy, and enables faster data collection.

Study population

The primary study population included chapatti samples sold by vendors at Shilabela market, and the secondary study population included the chapatti vendors operating in Shilabela market

Study area

The study was conducted in a few selected Chapatti stalls located within Shilabela Market, Geita District, Tanzania. It ran in 30 days of June 2025.

Sample size determination

To calculate the minimum sample size, the formula below was used by Kish and Leslie (2000) (Althubaiti, 2022).

Using the formula, $N = \frac{z^2pq}{d^2}$ (Kish, Leslie. 1965)

Where:

N = required sample size

Z = standard normal value at 95% confidence level = 1.96

P = estimated prevalence of bacterial contamination in street-vended foods, assumed at 75% (0.75) based on previous similar studies and expert assumption

q = 1 - p = 0.25

d = margin of error (precision) = 0.08 (8%)

Substituting the values Hence $N = (1.96^2 \times 0.75 \times 0.25) / 0.08^2$

= 112 respondents. Thus, the sample size was determined to be 112 chapatti samples.

Sampling technique

Convenient sampling was done during the selection of Chapatti from vendors. This sampling technique was chosen because of the limitations due to the number of available stalls at the study site.

Inclusion criteria

- Already made chapatti.
- Stalls for which their owners verbally consented to participate in the study

Exclusion criteria

- Raw materials (raw chapatti).
- Quality assurance and quality control

Data quality was ensured through carrying out analysis following a standardised analytical technique. The data collected was thoroughly checked for completeness. All the steps were guided by a qualified laboratory technician and an institution supervisor. Other quality controls observed were:

Pre-examination

Proper sample collection with minimised contamination was observed, i.e., sterilisation of sample bags, double

packaging, and disinfection of hands before sample handling.

A cool box was used in sample transportation to the laboratory.

Proper sample labelling for easy identification.

Proper storage of samples in the refrigerator at 4 to 8°C.

Examination

Sample preparation was done on arrival at the laboratory or within a time span of 24 hours.

Sample verification before examination.

Aseptic microbiological techniques were observed during analysis.

Proper care and maintenance of equipment, e.g., incubator temperature maintenance log charts, were carefully observed.

Followed and adhered to protocol SOPs. Post Examination

Verification of microbiological test results.

Recording of results.

Analysis, interpretation, and inference of results.

Writing of reports and documentation

Materials

A sterile normal saline solution to immerse the samples was prepared. Primary media, i.e., MAC, MSA, and BEA, onto which the samples will be cultured, peptone water, and NA for sub-culturing. Coagulase, Catalase, citrate, TSI, SIM, and Kovac's reagent for biochemicals, followed by NA and antibiotic discs for AST (Antimicrobial Susceptibility Testing), were also used.

Sample collection

Samples of 14 bags of different chapatti ingredients (approximately 1g of each) were collected from a random selection of different vendors. Generally, sample collection involved the following steps:

Sterile polythene bags were bought from shops, and surface sterilisation using 70% ethanol will always be done to prevent possible external contaminants.

The bags were double-packaged and transported to the sample collection site.

Disinfection of the hands using 70% ethanol was constantly done before handling the specimens, which were aseptically double packaged in sterile polythene bags.

The samples were then transported back to the laboratory in a cool box at 4°C to 8°C.

Laboratory analysis

Sample preparation

During sample preparation for analysis:

Using a sterile spatula, a spoonful of each sample was transferred to 10 mL of sterile normal saline in sterile tubes.

The tubes were covered with sterile aluminium foil and incubated at 37°C for 24 hours.

After inoculation on primary cultures, subculturing on NA was done.

Actual bacteria isolation

This followed the following steps: Different Gram staining forms from NA sub-cultures and different biochemical tests were carried out to identify the bacterium isolate to the species hierarchy. District Laboratory Practices 2 was used to identify the different bacteria isolated according to their biochemical tests.

Antimicrobial susceptibility patterns of the isolates

During this process, NA plates were prepared, and the Kirby-Bauer disc diffusion method of AST shall be used to determine the antimicrobial susceptibility patterns of the different isolates. The AST chart was used to interpret the zones of clearance. The different antibiotics included: tetracycline, Cefoxitin, Levofloxacin, Gentamycin, erythromycin, and linezolid.

Data management

All data that was collected in the study was handled in ways that allowed for data accuracy, integrity, and confidentiality. Specifically, the following steps were taken:

Sample identification where each chapatti sample was assigned a unique identification code in order to trace each sample and avoid errors in identification during analysis in the laboratory.

Lab records where microbiological data, including type of bacterial isolates and antibiotic susceptibility test results, were recorded in an organised and systematic way in laboratory logbooks, where it was then transferred into a Microsoft Excel spreadsheet for organisation and storage.

Questionnaires where data from chapatti vendors collected on hygiene and food handling were coded and entered into Excel for analysis.

Data backup where all electronic data was backed-up to a not accessible to the public password protected external storage device and cloud storage to prevent loss of data.

Data analysis

Descriptive statistics

Frequencies and percentages were calculated to show the prevalence of the different bacterial species isolated from the chapatti samples.

Summary tables were created to show the number and percentage of samples that were contaminated with each bacterial species.

Antibiotic susceptibility patterns

Results from the Kirby-Bauer disk diffusion method were interpreted according to CLSI standards.

Each bacterial isolate was classified either as sensitive (S), intermediate (I), or resistant (R) to each of the antibiotics used.

Excel was used to calculate the percentage of resistance for each antibiotic across all isolates, and the rate of multi-drug resistance (MDR) was determined by counting the number of isolates that are resistant to three or more classes of antibiotics.

Data presentation

Data were summarised into tables, bar graphs, and pie charts using Excel to help visualise and interpret the findings.

Narratives accompanied the visuals to more clearly describe the trends/patterns and relationships seen in the data.

All analyses were summarised to address the study objectives and to discuss any public health relevance of the findings.

Ethical considerations

Ethical approval for the study was obtained from the Faculty of Health Sciences, University of Kisubi. An introductory letter was provided to the management of Shilabela Market, Geita District, Tanzania, and all study procedures were thoroughly explained to the participants.

Informed consent was obtained from every vendor before participation, and all data will be anonymised to ensure confidentiality. Participants were informed of their right to withdraw from the study at any time without any penalty.

Results

The isolation and identification of bacterial pathogens from chapatti collected from Shilabela Market, Geita District, Tanzania.

Sample collection

112 chapatti samples were aseptically collected from several vendors at the Shilabela Market. Aseptic collection of 1g of each sample was achieved using sterile spatulas along with the polythene bags pre-treated with 70% ethanol. To prevent any contamination from the environment or otherwise, chapatti samples were double-packaged.

The samples were placed in a cool box with a temperature of 4 - 8 °C and transported for further microbiological examinations to the Sakamu Hospital Microbiology Laboratory. The samples were processed within 24 hours to ensure that the bacteria were still viable.

Primary culture

Each chapatti sample was homogenised in sterile normal saline (Normal Saline). Aliquots of the samples were inoculated into three selective and differential primary culture media:

MacConkey Agar (MAC), which is selective for Gram-negative enteric bacteria. It differentiates lactose fermenters (pink colonies) from non-lactose fermenters (no colour).

Mannitol Salt Agar (MSA) is selective for Staphylococcus species that ferment mannitol (yellow colonies).

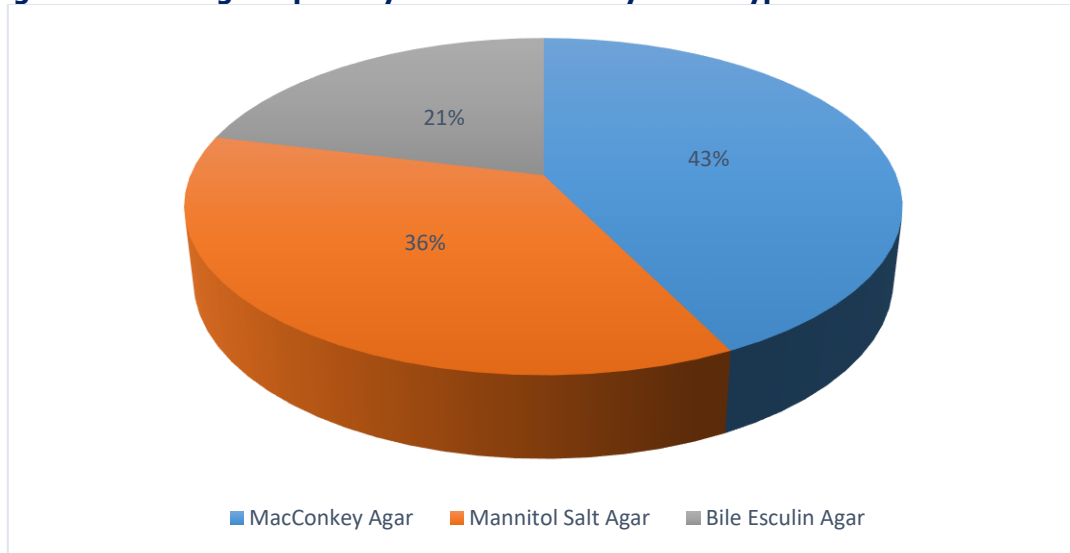
Bile Esculin Agar (BEA) is selective for Enterococcus and Group D Streptococci that hydrolyse esculin (black colouration).

The inoculated plates were incubated aerobically at 37 °C for 24 hours. Growth was recorded, and colony morphology, colour, and lactose or mannitol fermentation were noted.

Table 1: Results of primary culture media inoculation (N=112 samples)

Media Type	Number of Positive Samples	Percentage (%) Positive
MacConkey Agar	65	58.0%
Mannitol Salt Agar	55	49.1%
Bile Esculin Agar	32	28.6%

Figure 1: Showing the primary culture results by media type



From table 1 and figure 1, of the 112 chapatti samples cultured, 65 samples (58.0%) exhibited growth on MacConkey Agar, indicating the presence of Gram-negative enteric bacteria. Mannitol Salt Agar showed positive growth in 55 samples (49.1%), suggesting Staphylococcus species. Positive hydrolysis was exhibited in 32 samples (28.6%) on Bile Esculin Agar, which suggested the possible presence of Enterococcus or Group D Streptococci. Growth on more than one of the types of media indicates that these samples contained mixed bacterial populations.

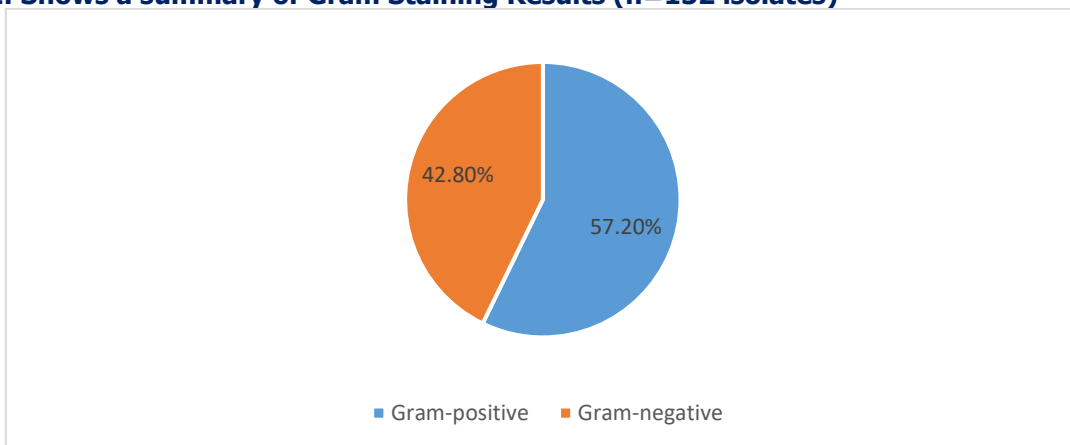
Aseptic distinct colonies were picked from primary plates and subcultured onto Nutrient Agar plates for purification and further analysis. The NA plates were then incubated at 37°C for 24 hours to allow for pure colonies.

Gram Staining of Subculture

The pure colonies obtained on NA were subsequently subjected to Gram staining, allowing for classification of bacteria into Gram-positive and Gram-negative groups, based on the characteristics of their cell wall

Subculture onto Nutrient Agar (NA)

Figure 2: Shows a summary of Gram Staining Results (n=152 isolates)



Out of 152 pure bacterial isolates (which totaled the positive cultures from all three media), 87 isolates (57.2%) were Gram-positive, largely from subcultures extracted from the Mannitol Salt Agar and the Bile Esculin Agar. The remaining 65 isolates (42.8%) were Gram-negative and were entirely drawn from subcultures utilising MacConkey Agar. This pattern followed the unique selective ability of the respective cultural media.

Biochemical identification of isolates

Following established protocols of use, biochemical tests were performed to identify bacterial species. Tests conducted included Coagulase, Catalase, Citrate

utilisation, Triple Sugar Iron (TSI) test, Sulfide Indole Motility (SIM), and Kovac's reagent for indole production.

The main bacterial species identified among the isolates were

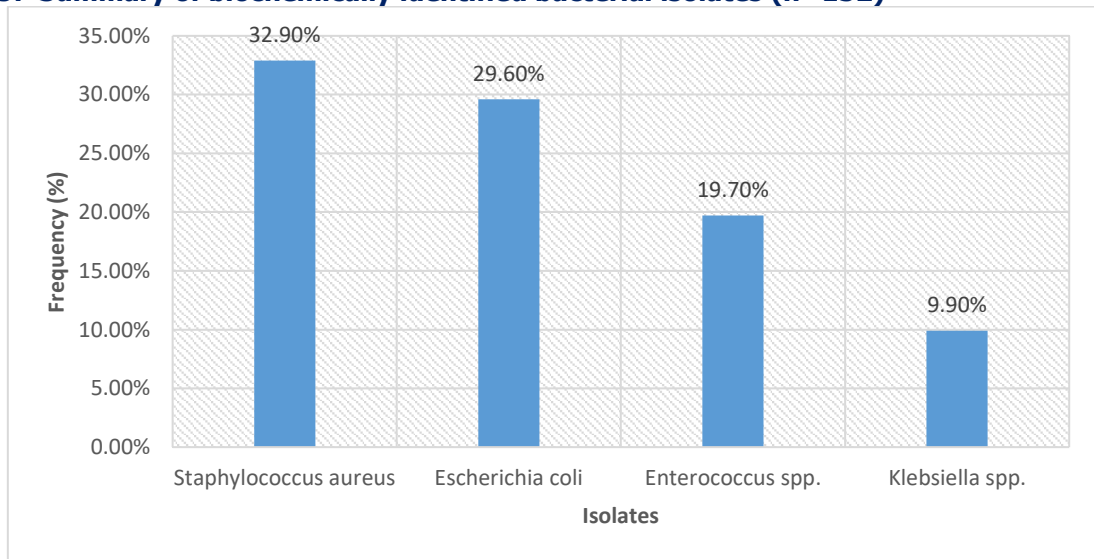
Staphylococcus aureus (Coagulase positive, Catalase positive, mannitol fermenter)

Escherichia coli (Lactose fermenter on MAC, Indole positive, Citrate negative)

Enterococcus spp (Esculin positive on BEA, Catalase negative)

Klebsiella spp (Lactose fermenter on MAC, Indole negative, Citrate positive)

Figure 3: Summary of biochemically identified bacterial isolates (n=152)



From table 3 and figure 2, the standard isolation and identification tests for bacteria show that *Staphylococcus aureus* was the most common isolate at 32.9%, and *E. coli* was second at 29.6%, followed by *Enterococcus spp.* (19.7%) and *Klebsiella spp.* (9.9%). This indicates a particular contamination profile for bacteria in chapatti sold at Shilabela Market.

Discussion

The study showed that the chapatti samples had four major bacterial pathogens present in chapatti samples collected from Shilabela Market: *Staphylococcus aureus* (32.9%), *Escherichia coli* (29.6%), and *Enterococcus spp.* (19.7%) and *Klebsiella spp.* (9.9%). This demonstrates critical food safety and hygiene issues that have significance for public health in the market. *Staphylococcus aureus* was the most common pathogen isolated. *S. aureus* is commonly involved in foodborne illness, especially during the preparation and storage of food. In a study by Somda et al. (2024), *S. aureus* was isolated from 70.83% of street food samples, clearly indicating the prevalence of *S. aureus* is significant in street foods (Somda et al., 2024).

Escherichia coli, a faecal indicator bacterium, was the second most common isolate. Its presence indicated faecal contamination and possibly less than adequate sanitary

and hygienic practices in the preparation stages. Nag et al. (2021) indicated that *E. coli* contamination in street foods was a high risk for public health in relation to food safety practices, especially in areas with limited sanitation and clean water access.

Enterococcus spp. and *Klebsiella spp.* were present in lower numbers. *Enterococcus spp.* are resilient in various environments and can indicate faecal contamination. *Klebsiella spp.* are recognised as opportunistic pathogens typically associated with immunocompromised individuals and are routinely associated with poor hygiene practices. The high level of these pathogens in chapatti sold at Shilabela market demonstrates a high degree of bacterial contamination, in relation to inadequate hygiene and sanitation practices. A scoping review published in the year 2023 demonstrated the prevalence of antimicrobial-resistant pathogens in ready-to-eat foods and thereby improved the safety of food (Onohuean et al., 2025).

Conclusion

In this study, bacterial pathogens have been isolated and identified from chapatti sold at Shilabela Market, Geita District, with a contamination profile dominated by *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus spp.*, and *Klebsiella spp.* The isolation of these bacteria,

many of which are known causative agents of food-borne illness, indicates potential health risks associated with the consumption of chapatti from this market.

Recommendations

Food vendors operating in the Shilabela Market should participate in regular and thorough training programs related to food safety that improve their knowledge of hygiene practices and reduce the potential risk of bacterial contamination in chapatti products.

Health authorities must regularly inspect street food vendors and conduct unannounced spot checks to assess the vendors' adherence to sanitisation and food handling regulations with the intent to improve food safety.

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List of abbreviations and acronyms

AST:	Antimicrobial Susceptibility Testing
MDR:	Multi-Drug Resistant
CLSI:	Clinical and Laboratory Standards Institute
NA:	Nutrient Agar
MSA:	Mannitol Salt Agar
MAC:	MacConkey Agar
TSI:	Triple Sugar Iron

Source of funding

The study was not funded

Conflict of interest

The author did not declare any conflict of interest

Data availability

Data is available upon request

Author contribution

John Kakubi collected data and drafted the manuscript of the study

James Kasozi supervised the study

Habert Mabonga supervised the study

Author biography

John Kakubi is a bachelor's degree student of biomedical laboratory technology at the University of Kisubi.

James Kasozi is a supervisor at the University of Kisubi

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