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Microbial Contamination and Antimicrobial Resistance in Ready-to-Eat Street Foods: Evidence from Nyamirambo–Biryogo, Rwanda

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Abstract

Background: Prepared food sold by street hawkers is vital to nutrition and economy in East African cities, but poor hygiene, infrastructure, and regulation heighten health risks. **Methods:** This study evaluated microbial contamination, physicochemical properties, and antimicrobial resistance of ready-to-eat foods from street vendors in Nyamirambo-Biryogo, a densely populated Kigali settlement. From January 2026 to September 2026, a cross-sectional study analyzed 50 food samples in 13 categories during peak times. Microbiological tests included total plate count, coliform count, and identification of *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella spp.* via ISO methods. Antimicrobial susceptibility and physicochemical tests (pH, water activity) were conducted. Kruskal-Wallis, ANOVA, and chi-square tests assessed statistical differences by food type and sampling time. **Results:** Overall, 72% of samples exceeded at least one East African Community (EAC/FS 03:2021) microbiological safety limit. Mean total plate count was $5.8 \pm 2.1 \log_{10}$ CFU/g, with highest contamination in Kachumbari (7.4), Brochettes (7.1), Fermented milk (6.9), and Chips (6.8). Total coliform count exceeded limits in 70% of samples. *Staphylococcus aureus*, *Salmonella*, and *Escherichia coli* were in 28, 36, and 12 percent of samples, respectively. Significant microbial load differences were found in midday versus morning and evening samples ($p = 0.028$). High moisture (water activity 0.95) and near-neutral pH supported pathogen growth. Multidrug resistance (MDR) was observed in 64% of *Escherichia coli* isolates mainly resistant to ampicillin, tetracycline, and amoxicillin-clavulanate and 67% of *Salmonella* isolates to resistant to ampicillin and tetracycline. **Conclusions:** Major microbial contamination of ready-to-eat foods in Nyamirambo-Biryogo

poses risks of food-borne diseases and antimicrobial resistance. Immediate actions are needed, such as food safety education, improved hygiene, temperature control, and regulatory structures to protect health and influence food safety policy in Rwanda.

Keywords: Ready-to-eat foods, street vendors, microbial contamination, antimicrobial resistance, food safety, Rwanda.

1. Introduction

Ready-to-eat (RTE) foods sold on the streets are a vital part of the food distribution system and a vital source of livelihood and food to millions of people in the developing world, especially in sub-Saharan Africa [1], [2]. With urbanization, there is increased consumption of street foods, which are healthy, lower priced, and accessible, and the food contributes significantly to the provision of affordable and convenient meals to the urban population, especially to those who have little time and resources to prepare meals at home [3], [4]. Although street-vended ready-to-eat foods have high socioeconomic value, they are very dangerous to the health of the population because of poor food handling habits and unhealthy environments that cause contamination with microorganisms. The microbiological safety of street foods in developing countries has been a long-standing international problem, because such a challenge threatens the health of consumers and must be solved with the combined efforts of all countries. More broadly, food safety failures carry substantial direct and indirect health and economic consequences, underscoring why microbiological monitoring of informally sold foods is a public health priority [5,6]. These foods are contaminated with microorganisms at different points in the food chain, during preparation and consumption, mainly because of poor hygiene and inadequate infrastructure in informal vending set ups. Stakeholders operating within such informal public markets in East Africa have themselves identified weak infrastructure and inconsistent oversight as core drivers of these food safety gaps [7]. Studies carried out in different countries in Africa and Asia have consistently shown alarmingly high concentrations of pathogenic microorganisms in street foods which in most cases are above the internationally set safety standards [8], [9].

Escherichia coli, *Salmonella spp.*, *Staphylococcus aureus*, and other bacteria are the most commonly found in ready-to-eat street foods. A comprehensive assessment of street foods at Khartoum Central Market revealed that all tested street-sold sandwich samples exceeded the total viable bacterial count limits set by regulatory authorities. This contamination was primarily due to poor food handling practices, inadequate personal hygiene, and insufficient sanitation facilities [10]. Similarly, research conducted in different regions, including Erbil, Iraq, and several African nations, has shown that street vendors consistently exhibit

higher rates of *Salmonella* contamination compared to regulated food establishments [11], [12]. The level of food safety awareness and the hygienic habits of street food vendors are crucial factors influencing the microbiological contamination of ready-to-eat foods. Numerous studies have indicated that many street food vendors, particularly in developing countries, possess limited formal education and lack adequate training in food safety and hygiene protocols [13], [14]. The poor handwashing, poor use of protective equipment, absence of a head covering, and direct hand contact with food are all practices of the vendor that are likely to cause pathogenic contamination of food. [13], [15].

Moreover, the lack of adequate infrastructure, such as the lack of access to clean water to prepare food, insufficient facilities to store food, and unsanitary waste disposal systems, adds to the problems of vendors adhering to food safety requirements [13], [16]. Poor knowledge, negative attitudes, and insufficient infrastructure inevitably result in the ideal environment that supports the growth of microbes and the spread of foodborne diseases, especially among the vulnerable groups of the population, such as children and immunocompromised people. The availability of antibiotic-resistant bacteria in the ready-to-eat foods sold at the street level is a new and rather alarming aspect of the food safety threats in developing nations. In recent reports, the prevalence rate of multidrug-resistant bacteria observed in street foods is high, and the pattern of resistance endangers the effectiveness of antibiotics frequently used [17], [18].

Strains of *Escherichia coli* producing Extended-Spectrum beta-lactamases (ESBL) are increasingly detected in ready-to-eat street foods, restricting treatment options for foodborne infections [15]. The spread of multidrug-resistant pathogens through the street food chain represents a critical One Health challenge, necessitating integrated surveillance and targeted intervention strategies [14,15,16].

While previous research has highlighted significant microbial contamination and inadequate hygiene in street food sold in parts of Kigali, there is a lack of specific evidence on how the daily food safety practices of vendors in Nyamirambo–Biryogo quantitatively affect microbial levels in various ready-to-eat foods and throughout the handling process. Specifically, there is limited understanding of how particular actions, such as water sourcing, utensil cleanliness, time and temperature management, salad preparation, and cross-contamination between raw and cooked foods, interact with local environmental conditions and infrastructure to influence contamination patterns in this densely populated, busy street-food area. There is a shortage of comprehensive assessments that simultaneously evaluate observed or reported vendor practices, environmental and infrastructural conditions (such as water, waste management, and stall design), and microbiological outcomes in ready-to-eat foods from Nyamirambo–Biryogo. Empirical data on the temporal dynamics of contamination, such as differences between morning, midday, and evening; weekdays versus weekends; and market event days in relation to holding times and ambient temperatures

at Nyamirambo–Biryogo stalls, is lacking. The role of specific food categories, including raw salads like kachumbari, high-protein foods such as brochettes and quick beans, and fermented products, in consumer risk has not been systematically compared using standardized microbiological criteria in this context [19], [20]. Information on the presence and patterns of antimicrobial-resistant bacteria in these street-vended foods, and their correlation with upstream factors like the sourcing of meat and milk or vendors' knowledge and perceptions of antimicrobial use, remains limited in the Nyamirambo–Biryogo area. This highlights a clear need for a comprehensive, mixed-methods assessment that connects observed food-handling and safety practices of Nyamirambo–Biryogo street vendors with detailed microbiological analysis of ready-to-eat foods, including antimicrobial resistance profiling, across different times of day and food types. Addressing this gap will provide actionable, location-specific evidence to guide targeted regulations, training, and infrastructural improvements aimed at reducing foodborne and antimicrobial-resistance risks associated with ready-to-eat street foods in Nyamirambo–Biryogo, Nyarugenge District. This study aimed to assess food safety practices and the level of microbial contamination in ready-to-eat (RTE) foods sold by street vendors in Nyamirambo–Biryogo, Nyarugenge District, Rwanda. The study's research questions were as follows:

- a) What is the current state of hygiene and sanitation, including water sources, waste management, stall cleanliness, and equipment hygiene, at street-food vending locations in Nyamirambo–Biryogo?
- b) What are the levels of microbial contamination, such as total plate count, coliforms, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella spp.*, in selected ready-to-eat foods sold by street vendors in Nyamirambo–Biryogo?
- c) How do the food safety practices of vendors and the environmental conditions affect the microbial quality of ready-to-eat foods in Nyamirambo–Biryogo?

This research aimed to determine the microbiological contamination levels, antimicrobial resistance patterns, and physicochemical characteristics of ready-to-eat foods sold by street vendors in Nyamirambo–Biryogo, to produce evidence to support specific population health interventions. This study did not directly evaluate the vendor's hygiene practices and food-handling behaviour, which are proposed for future studies [13], [21]. This would create important information to protect consumer health and enable a sustainable change within the informal food sector. The results would add to the current literature on the safety of street food in sub-Saharan Africa and offer practical information to local health departments to establish evidence-based food safety guidelines and training on street food vendors.

2. Materials and Methods

2.1 Study Design and Setting

The study utilizes a cross-sectional descriptive design to assess food safety practices and levels of microbial contamination in ready-to-eat foods from various vendors at a single point in time. This method is ideal for determining prevalence without suggesting causation, enabling effective data collection on vendor behaviors, environmental conditions, and contamination through observations, interviews, and laboratory tests. The study was conducted from January 2026 to September 2026 within Nyarugenge District of Kigali City, Rwanda, focusing on the densely populated informal settlement of Nyamirambo-Biryogo, with coordinates approximately 1.9582 °S, 30.0892 °E. This location is recognized as one of Kigali's most lively street food centers, revolving around Amarange Rest, an open-air communal dining space bustling with more than 60 stalls that operate regularly. This vibrant hub attracts crowds with its affordable ready-to-eat meals among the neighborhood's dense, informal urban landscape. Its unregulated yet energetic setup has heightened food safety challenges in this high-traffic area. The setting is characterized by a high volume of pedestrian traffic, inadequate infrastructure for safe food handling, and minimal formal food safety oversight, which are common traits of high-risk informal food systems in East Africa. The vendor stalls lack access to clean drinking water, proper handwashing facilities, refrigeration, and a reliable waste disposal system. Utilities are shared, and food is prepared in an open-air environment exposed to ambient temperatures, dust, insects, and environmental pollution.

2.2 Sampling Strategy and Population of the Study

The research utilized purposive sampling to focus on ready-to-eat foods that present the greatest microbiological hazards, such as grilled meats, fried snacks, and salads, with an emphasis on those popular among locals, in accordance with established methods in street food studies in Sub-Saharan Africa [22], [23]. This non-probability approach enabled targeted evaluation of significant safety issues without expending resources on less pertinent samples. To ensure a balance between practicality, the capacity of local labs for microbial analysis, and the statistical power required to detect moderate variations among food categories, a sample size of $n=50$ was selected. This decision also considered the limited number of functioning restaurants and stalls, around 60, in Nyamirambo-Biryogo, aiming for the highest possible representativeness given the resource constraints. The Cochran formula for determining sample size was employed to calculate the sample size and is expressed as

$$n = \frac{N}{1 + N(e)^2} \quad (1)$$

Where n = sample size, N =total population of restaurants, and e = margin of error

$$n = \frac{58}{1 + 58(0.05)^2}$$

Traditionally, the Cochrane formula estimates proportions in human populations, not food samples. Here, it approximated stalls (N = 58) to a manageable sample size; the result (n = 50) aligns with past food safety studies in East Africa (30 to 60 samples). However, sub-sample sizes per stratum (n = 12) are small, limiting statistical power, as noted in Section 3.6. Sampling was stratified by three peak consumption periods, considering food freshness, temperature, and vendor activity: Morning (07:00-09:00 CAT) for breakfast/snacks with fresh food and cooler temperatures; Midday (12:00-13:00 C). Sampling was done without informing vendors to maintain ecological validity and reduce observer bias (Hawthorne effect). Purchases used local currency (Rwandan Francs) to reflect real consumer behavior and minimize vendor behavior changes. No special requests or identifications were made that could affect normal food handling.

2.3 Food Choice Standards and Risk Classification

Thirteen ready-to-eat (RTE) foods were chosen based on several criteria: (i) the frequency of sales by five vendors of each food item, (ii) customer patronage, with more than ten purchases per hour during reconnaissance surveys, and (iii) the composition and preparation methods, including carbohydrate-based, protein-based, dairy-based, and raw vegetable-based foods. This selection of 13 foods represents over 90 percent of the daily sales volume at the study location. The risks associated with these foods were evaluated following the East African Community (EAC) food safety guidelines (EAC/FS 03:2021), which involved analyzing factors such as moisture content, pH levels, protein load, potential for post-cooking contamination, and time-temperature abuse. The foods were then categorized into risk levels: low, medium, medium-high, high, or very high. (Table 1).

Table 1. Food types sampled: descriptions and risk categorisation

Food type	Description / typical preparation	Risk category	Basis for risk assignment
Ugali	Stiff porridge of maize flour and boiling water	Low–Medium	Low moisture (aw < 0.85); heat-processed; consumed hot.
Isombe stew	Cassava leaves cooked with oil, onions, and eggplant	Medium	Plant-based; post-cooking contamination risk; extended holding time.
Plain rice	Boiled rice, often held >2 h at ambient temperature	Medium	Cooked starch supports bacterial growth if held improperly.
Quick beans	Pre-cooked beans reheated rapidly on-site	Medium–High	Protein-rich; frequently kept warm for prolonged periods; reheating adequacy uncertain.
Chips (fries)	Deep-fried potatoes, served hot or warm	High	High fat + moisture content; post-frying handling increases pathogen risk.
Boiled eggs	Hard-boiled, peeled on-site; manually handled	Low–Medium	Intact shell provides protection; peeling introduces direct contamination risk.

Brochettes	Skewered goat/beef, grilled over charcoal; held ≥ 1 h	High	Protein-rich; cross-contamination from handling; uncertain charcoal grill hygiene.
Fermented milk (Ikivuguto)	Traditionally fermented, unpasteurised cow milk	High	Raw dairy; natural fermentation without heat step; time-dependent safety.
Kachumbari	Fresh chopped tomato, onion, and chili; no cooking	Very High	Raw produce; high moisture; neutral pH; high likelihood of fecal contamination.
Samosa	Deep-fried pastry filled with meat or vegetables	High	Filling may be undercooked; post-frying handling and storage risks.
Mandazi	Fried dough (yeast/sugar), served warm	Medium	Low protein; oil absorption increases thermal stability; extended holding risk
Matoke	Steamed green banana, peeled and served hot	Low–Medium	High heat kill-step; consumed immediately; minimal post-cooking handling.

Note: Risk categories based on EAC/FS 03:2021 criteria: moisture content, pH level, protein load, post-cooking handling intensity, and time–temperature exposure. aw, water activity; VH, very high; H, high; M-H, medium–high; M, medium; L-M, low–medium.

2.4 Sample Collection and Chain of Custody

A total of 50 samples were used during the three time periods, the number being proportional to the frequency of consumption or risk profile. The foods identified to be at a higher risk (including Kachumbari, Brochettes, Fermented milk) were given a higher sampling weight in order to make sure that the most at-risk categories will be adequately represented (see Table 2).

Table 2. Sampling distribution across food types and time periods

Food type	Morning (n)	Midday (n)	Evening (n)	Total (N)	Rationale for sampling weight
Chips	2	2	2	6	Highly popular throughout the day; frequent reheating increases risk
Brochettes	2	2	2	6	Top-selling protein item; long holding times increase contamination risk
Plain rice	2	2	1	5	Staple with high demand, especially midday
Quick beans	2	2	1	5	Frequently paired with rice; peak consumption midday
Fried snacks mix	2	2	1	5	Popular evening snack; mixed preparation elevates risk
Ugali	1	2	1	4	Common at breakfast and dinner; moderate risk profile

Kachumbari	1	2	1	4	High-risk raw food; additional midday sampling allocated
Isombe stew	1	1	1	3	Less frequently consumed; baseline microbial assessment
Boiled eggs	1	1	1	3	Low–moderate risk; routine monitoring
Samosa	1	1	1	3	High-risk fried snack; consistency checking across sampling times
Mandazi	0	1	1	2	Primarily a midday/evening snack
Matoke	0	1	1	2	Served hot; lower risk; minimal breakfast sales
Fermented milk (Ikivuguto)	0	1	1	2	Daytime-only product; peak vendor activity midday
Total	15	20	15	50	—

Note: Samples collected across three peak consumption periods: morning (07:00–09:00 CAT), midday (12:00–13:00 CAT), and evening (18:30–19:00 CAT). Higher-risk food categories received proportionally greater sampling weight to ensure adequate representation. N, number of samples.

The mass of the samples was 100 g (dense items: ugali, brochettes), 200 g (heterogeneous items: kachumbari, fried snacks mix), to be sure of having a sufficient amount of material to replicate the analyses. All samples were identified using an alpha-numeric number (e.g., RC29 Rice sample #29, FM31 Fermented Milk sample #31, FS46 Fried Snacks sample #46) that combined sample type, sequence, and time of collection. All samples were put in sterile, food grade, polyethylene containers (250 mL capacity), labeled in advance with the following: unique sample ID, date of collection, time of collection (time was recorded to the minute), ambient temperature of point of purchase (measured using calibrated infrared thermometer, Fluke 62 Max+) and stall identifier (e.g., BIR-07 was a stall at Biryoko). Right after purchase, samples were stored in tested insulated coolers with phase-change ice packs (to keep the 4-hour internal temperature at 2–6 °C). Data loggers (Elitech RC-51H) were used to record temperature within coolers at 15-minute intervals throughout transit. Temperature logs confirmed that all samples were maintained at 2–6°C throughout transport; no cold chain excursions above 6°C were recorded for any sample batch. The samples were transported to the Rwanda Standards Board (RSB) Food Microbiology Laboratory (which is ISO/IEC 17025:2017 accredited) within the period of 60 minutes of collection. The start of all laboratory analyses was within 24 hours of collection, which is in accordance with ISO microbiological standards.

2.5 Sample Analysis

2.5.1 Sample Preparation and Processing

In sterile stomacher bags, for each sample, 25 g were weighed in a sterile stomacher bag and homogenised with 225 mL of Buffered Peptone Water (BPW) using a Lab-Blender 400 for 60 seconds, giving a 1:9 (w/v) ratio equivalent to the standard 1:10 dilution factor (ISO 6887-1:2017). Samples underwent 10-fold dilution with sterile 0.1% peptone water. Microbiological tests followed ISO standards at the Rwanda Standard Board. Total Plate Count: Dilutions were spread on PCA, incubated at 30°C for 72h. Colonies (30-300 CFU) were recorded as log₁₀ CFU/g. Total Coliform Count: Dilutions were spread on VRBL agar, incubated at 37°C for 24-48h. Red colonies were counted as coliforms (log₁₀ CFU/g).

2.5.2 Bacterial Isolation, Detection and Characterisation

Escherichia coli Detection: A 0.1 mL aliquot of each sample dilution was inoculated onto TBX agar medium and incubated at 44°C for 24 h to detect and quantify *Escherichia coli*, in accordance with ISO 16649-2:2001 (note: although ISO 16649-2 recommends 44°C, some protocols use 37°C for TCC and 44°C specifically for thermotolerant *Escherichia coli*; the 44°C temperature was applied here specifically for *Escherichia coli* enumeration). Blue colonies were counted after β-glucuronidase testing (log₁₀ CFU/g). *Staphylococcus aureus* Detection: A 0.1 mL aliquot of each dilution was inoculated onto Baird-Parker agar and incubated at 37°C for 24–48 h to detect and quantify *Staphylococcus aureus*. Black colonies with clear zones were confirmed through Gram staining and coagulase testing (log₁₀ CFU/g). *Salmonella* spp. Detection: A 25 g sample was pre-enriched in Buffered Peptone Water (BPW; 37°C, 18 h), followed by selective enrichment in Rappaport-Vassiliadis Soya (RVS) broth (41.5°C, 24 h); a 10 µL loopful of the enrichment culture was then streaked onto XLD and HE agars, with colonies confirmed using TSI, urease, and lysine decarboxylase tests. Results were reported as Present/Absent per 25 g.

2.6 Physicochemical Analyses

pH Measurement: AOAC Method 981.12 used a calibrated Hanna HI2211 digital pH meter to measure pH. A 1:1 homogenate was prepared by combining 10 g of the sample with 10 ml of sterile distilled water and letting the samples equilibrate (15 minutes). Each day, the pH meter was calibrated using standard buffers (pH 4.01, 7.00, and 10.01). Water Activity (aw): Water activity was determined at 25°C with an Aquelab Series 4TE meter, where three standards were used: Lithium chloride (aw = 0.11), sodium chloride (aw = 0.75), and potassium sulfate (aw = 0.97). Antibiotic Susceptibility Testing: *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus* isolates were tested using the Kirby-Bauer disk diffusion method. Cultures cultured on Mueller-Hinton agar were brought up to a 0.5 McFarland standard and inoculated on plates. Antibiotic disks, which included ampicillin (10 µg), amoxicillin/clavulanic acid (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (1.25/2 This panel was chosen because: (1) it can be found on the WHO List of Critically Important Antimicrobials to Human Medicine (2019); (2) it is commonly used in Rwandan human and veterinary medicine; (3) it is

recommended on the CLSI M100-Ed33 list in *Enterobacteriaceae* and *Staphylococcus spp*; (4) it is a problem. Inhibition zones were interpreted as recommended by CLSI after incubation at 35-37 °C, 18-24 hours. Quality Assurance and Quality Control: QA/QC procedures were based on ISO/IEC 17025: 2017. Media Validation: Sterility tests and growth promotion tests with reference strains were done. Process Controls: Each analytical batch contained: (i) a field blank - sterile BPW container opened and reclosed at the sampling site without coming into contact with food to determine airborne or handling contamination during field work; (ii) a laboratory blank - sterile. Blinding of Analysts: The identifiers of samples were eliminated in the enumeration process to reduce bias. Instrument Maintenance: All instruments had their calibration records that were observed as per the manufacturer's instructions.

2.7 Data Management and Statistical Analysis

The data was entered in Microsoft Excel 2019, using automatic field alignment for inconsistencies. Analysis was done using IBM SPSS Statistics v25 and R 4.3.1 with dplyr, ggplot2, and rstatix packages. Microbial counts were log₁₀ transformed before parametric tests. Descriptive statistics were calculated for each parameter (13 categories) by food type and sampling time (3 categories). The Shapiro-Wilk test assessed normality at 0.05. One-way ANOVA tested food categories with normality ($W p > 0.05$); otherwise, the Kruskal-Wallis H test was used for non-normal distributions ($p < 0.05$). Of the 13 food categories, Matoke, Boiled eggs, Ugali, and Mandazi (all $p > 0.05$ of TPC) were normative, while 9 categories required non-parametric tests. One-way ANOVA compared groups when normality conditions were met; otherwise, the Kruskal-Wallis test was used, with post-hoc comparisons via Tukey HSD or Dunn tests. A similar analysis was conducted for sampling time. *Salmonella* presence was analyzed using Chi-square or Fisher's exact test. Statistical significance was set at 0.05. Visualization depicted boxplots of log₁₀ CFU/g, compliance bar charts, and microbial contamination heat maps. Raw data was kept in Excel for transparency.

2.8 Regulatory Compliance and Ethics

Although this study did not involve human subjects or biological samples, ethical approval was sought from the Rwanda National Ethics Committee (RNEC) (reference: 123/CAF/2026, approved on 23 January 2026) to ensure compliance with national research governance requirements, which mandate institutional oversight for all field-based research conducted in public settings. Since the study involved food sample collection and environmental observation only, individual vendor consent was not required per national guidelines; however, the study protocol was reviewed and approved by the Nyarugenge District authorities.

3. Results

3.1 Microbiological quality of street-vended ready-to-eat foods

In Nyamirambo-Biryogo, 50 ready-to-eat (RTE) food samples from 13 different categories were collected from street vendors and subjected to detailed microbiological analysis (see Table 2 in Section 2.4 for

sampling distribution). Overall, 36 samples, accounting for 72%, exceeded one or more microbiological safety limits as defined by the East African Community Standard EAC/FS 03:2021. The total plate count (TPC) for all samples averaged $5.8 \pm 2.1 \log_{10}$ CFU/g, with a range from 4.2 to 7.4 $\log_{10}$ CFU/g. The mean total coliform count (TCC) was $4.5 \pm 2.3 \log_{10}$ CFU/g. Contamination levels were notably high, with *Escherichia coli* found in 12% (6/50), *Staphylococcus aureus* in 28% (14/50), and *Salmonella spp.* in 36% (18/50) of the samples. The microbial counts for these pathogens in positive samples were concerning: *Escherichia coli* ranged from 2.3 to 3.8 $\log_{10}$ CFU/g; *Staphylococcus aureus* counts in positive samples varied between 2.1 and 4.5 $\log_{10}$ CFU/g, significantly exceeding the EAC/FS 03:2021 limit of 2.0 $\log_{10}$. The EAC/FS 03:2021 requirements classify risks in terms of the content of moisture, pH, protein, and preparation means, and handling intensity. The three-fold sampling was done in order to record the variation in the patterns of contamination with time. The overall microbiological and physicochemical parameters of each food sample, such as total plate counts (TPC), total coliform counts (TCC), presence of pathogens, pH, water activity, and classification of risks, are indicated in Table 3.

Table 3. Microbiological and physicochemical characteristics of ready-to-eat foods from street vendors in Nyamirambo–Biryogo (n = 50)

Food type	n	TPC ($\log_{10}$ CFU/g)	TCC ($\log_{10}$ CFU/g)	<i>Escherichia coli</i> (%)	<i>Staphylococcus aureus</i> (%)	<i>Salmonella</i> (%)	pH (mean $\pm$ SD)	aw (mean)	Non-compliant (%)	Risk
Kachumbari	4	7.4 ± 1.2	8.1 ± 0.9	100	50	25	5.8 ± 0.3	0.98	100	VH
Brochettes	6	7.1 ± 1.4	6.9 ± 1.1	50	67	33	6.2 ± 0.4	0.96	100	H
Fermented milk	2	6.9 ± 0.8	5.5 ± 0.6	0	100	50	4.1 ± 0.2	0.99	100	H
Chips	6	6.8 ± 1.3	5.2 ± 1.0	33	30	20	6.4 ± 0.3	0.95	100	H
Quick beans	5	6.5 ± 1.1	5.8 ± 0.9	40	60	40	6.7 ± 0.2	0.97	100	M-H
Samosa	3	6.2 ± 0.9	4.8 ± 0.7	33	33	33	6.1 ± 0.3	0.92	100	H
Plain rice	5	5.6 ± 1.0	3.9 ± 0.8	20	20	20	6.5 ± 0.2	0.94	80	M
Fried snacks mix	5	5.4 ± 1.2	3.6 ± 0.9	0	20	40	6.3 ± 0.3	0.89	80	M
Isombe stew	3	5.2 ± 0.9	3.1 ± 0.5	0	33	33	6.4 ± 0.2	0.91	67	M
Ugali	4	4.9 ± 1.3	2.5 ± 0.6	0	5	0	6.8 ± 0.2	0.82	50	L-M
Mandazi	2	4.8 ± 0.8	2.6 ± 0.5	0	0	20	6.6 ± 0.2	0.88	50	M
Boiled eggs	3	4.6 ± 1.1	2.8 ± 0.7	0	0	0	7.0 ± 0.2	0.96	33	L-M
Matoke	2	4.2 ± 0.9	2.3 ± 0.4	0	0	0	6.9 ± 0.2	0.93	0	L-M

TPC, total plate count (log CFU/g); TCC, total coliform count (log CFU/g); aw, water activity. Categories of risks: VH, very high; H, high; M-H, medium-high; M, medium; L-M, low- medium. EAC/FS 03:2021 limitations TPC 5.0 log CFU/g; TCC 2.0 log CFU/g; *Escherichia coli* and *Staphylococcus aureus* 100 CFU/g (2.0 log CFU/g); *Salmonella* not present/25g. Standard deviation is the difference between means. Percentages that are non-compliant denote samples that have a minimum of one microbiological threshold. Total plate count and general hygiene indicators.

3.1.1 Total plate count

The average total plate count (TPC) was recorded at $5.8 \pm 2.1 \log_{10}$ CFU/g. Among the foods tested, Kachumbari exhibited the highest bacterial count at $7.4 \pm 1.2 \log_{10}$ CFU/g, followed by Brochettes at $7.1 \pm 1.4 \log_{10}$ CFU/g, Fermented milk at $6.9 \pm 0.8 \log_{10}$ CFU/g, and Chips at $6.8 \pm 1.3 \log_{10}$ CFU/g (Fig. 1). These values significantly exceeded the EAC/FS 03:2021 standard limit of $5.0 \log_{10}$ CFU/g ($p < 0.001$, as determined by a one-sample t-test against the regulatory benchmark). The Kruskal-Wallis test indicated substantial differences in TPC among the 13 food categories ($H = 32.4$, $df = 12$, $p < 0.001$). A Dunn test with Bonferroni correction revealed that Kachumbari, Brochettes, Fermented milk, and Chips had notably higher TPC levels compared to the lower-risk foods such as Matoke ($4.2 \pm 0.9 \log_{10}$ CFU/g), Boiled eggs ($4.6 \pm 1.1 \log_{10}$ CFU/g), Mandazi ($4.8 \pm 0.8 \log_{10}$ CFU/g), and Ugali ($4.9 \pm 1.3 \log_{10}$ CFU/g). Foods that were adequately heat-treated and subjected to minimal post-cooking handling maintained TPC values within the acceptable regulatory limits.

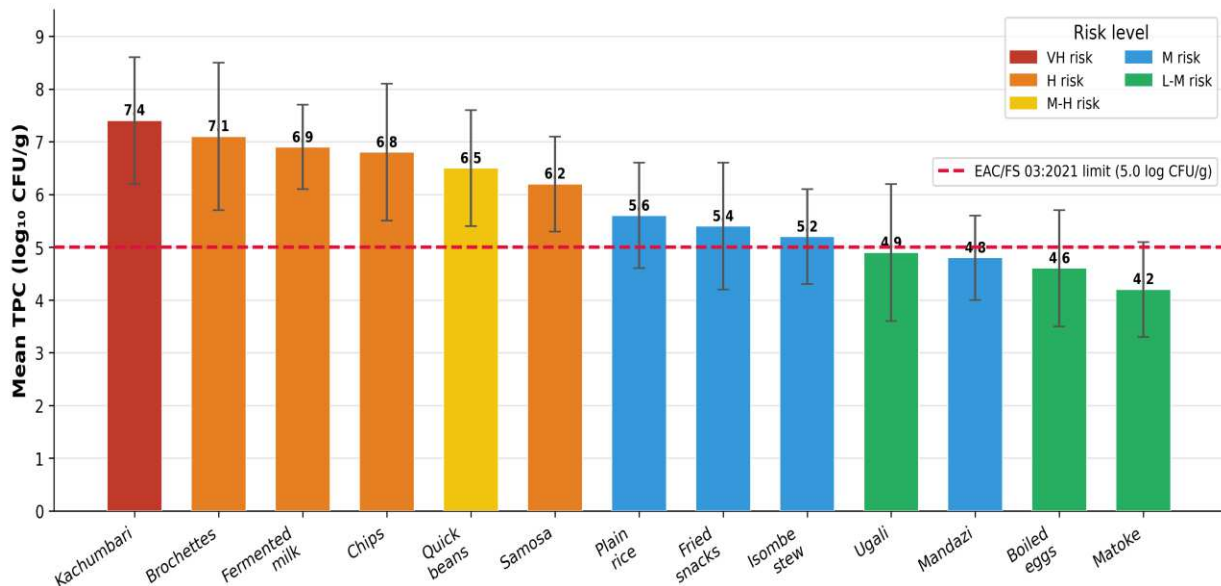


Fig. 1 Mean total plate count (TPC, log₁₀ CFU/g) by food type for ready-to-eat foods from street vendors

in Nyamirambo–Biryogo (n = 50). Error bars: standard deviation. Red dashed line: EAC/FS 03:2021 regulatory limit (5.0 log₁₀ CFU/g). Bar colours reflect the food risk category.

Distribution of the number of plates sold by street vending in Nyamirambo-Biryogo by 13 food types. Standard deviation is represented by error bars. Red dashed horizontal line implies acceptable limit of EAC/FS 03:2021 (5.0 log CFU/g). Colors of bars refer to the level of risk: red (very high risk), orange (high risk), blue (medium risk), green (low-medium risk). Kachumbari, Brochettes, Fermented milk, and Chips were way beyond the regulatory limit.

3.1.2 Total coliform count and fecal contamination indicators

The presence of total coliform bacteria is used as an indicator of overall sanitary quality, as well as the possible presence of fecal contamination in foodstuffs [24], [25]. The mean Total Coliform Count (TCC) for all samples was 4.5 ± 2.3 log CFU/g, with 70% (35 out of 50) of the samples exceeding the EAC/FS 03:2021 standard of 2.0 log CFU/g (Fig. 2). Kachumbari exhibited the highest coliform levels at 8.1 ± 0.9 log CFU/g, nearing 6 log units of contamination, indicating significant contamination from irrigation water, soil, or unwashed hands. Elevated TCC values were also noted in brochettes (6.9 ± 1.1 log CFU/g), Quick beans (5.8 ± 0.9 log CFU/g), Fermented milk (5.5 ± 0.6 log CFU/g), and Chips (5.2 ± 1.0 log CFU/g), likely due to cross-contamination of raw ingredients, poor hand hygiene, use of contaminated water during preparation or washing, and insufficient temperature control during storage. Foods with lower TCC included Matoke (2.3 ± 0.4 log CFU/g), Ugali (2.5 ± 0.6 log CFU/g), Boiled eggs (2.8 ± 0.7 log CFU/g), and Mandazi (2.6 ± 0.5 log CFU/g). ANOVA revealed significant differences in TCC among various food categories ($F = 18.7$, $df = 12, 37$, $p = 0.001$). Tukey's HSD post-hoc test showed that raw or minimally processed foods, as well as those with high protein content and extended ambient storage times, had notably higher coliform levels compared to freshly cooked, low-moisture, or heat-treated foods consumed immediately after cooking ($p < 0.05$ for relevant comparisons).

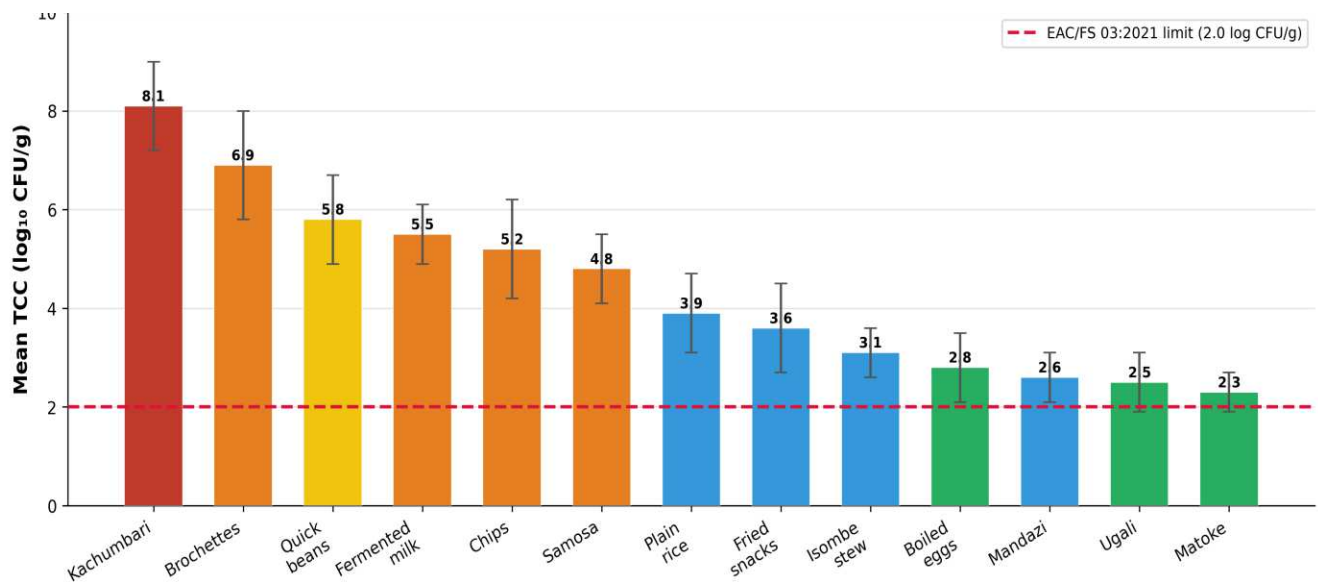


Fig. 2 Mean total coliform count (TCC, log₁₀ CFU/g) by food category, sorted by contamination level ($n = 50$). Error bars: standard deviation. Red dashed line: EAC/FS 03:2021 threshold (2.0 log₁₀ CFU/g). Kachumbari and other high-risk foods markedly exceeded the regulatory limit.

Mean values of total coliform count by category of foods with error bars of the standard deviation. Vertical red line is an indication of EAC/FS 03:2021 acceptable threshold (2.0 log CFU/g). Foods are placed in order of their contamination level. The quality of sanitation was poor, as Kachumbari and other high-risk foods were well above the regulatory limit.

3.1.3 Detection and prevalence of pathogenic bacteria

3.1.3.1 *Escherichia coli* contamination

While the overall prevalence of *Escherichia coli* was relatively low at 12% (6 out of 50), its presence in significant food categories highlights pathways of fecal contamination that differ by food type (Fig. 3). Every Kachumbari sample tested positive for *Escherichia coli* (4 out of 4, 100%), with concentrations ranging from 2.3 to 3.8 log₁₀ CFU/g (200-6300 CFU/g), likely due to contamination from irrigation water, unwashed hands, or dirty surfaces. In cooked foods, *Escherichia coli* was identified in half of the Brochettes (3 out of 6), 40% of Quick beans (2 out of 5), 33% of Chips (2 out of 6), and Samosa (1 out of 3), and 20% of Plain rice (1 out of 5). The detection of *Escherichia coli* in these cooked foods suggests either insufficient cooking temperatures (below 74 °C) or recontamination through handling or utensils. No *Escherichia coli* was found in Fermented milk, Fried snacks mix, Ugali, Isombe stew, Boiled eggs, Matoke, and Mandazi, likely due to heat treatment, low pH, deep frying, or immediate consumption. A Chi-square test showed a significant link between food category and *Escherichia coli* presence ($\chi^2 = 24.6$, $df = 12$, $p = 0.017$), with raw vegetable-based and inadequately heated protein items having a higher detection risk. It is important

to note that this study did not conduct molecular characterization for Shiga toxin-producing *Escherichia coli* (STEC) virulence genes (*stx1*, *stx2*, *eae*) due to resource limitations. Consequently, the pathogenic *Escherichia coli* isolates could not be further classified, which is acknowledged as a limitation and recommended for future research.

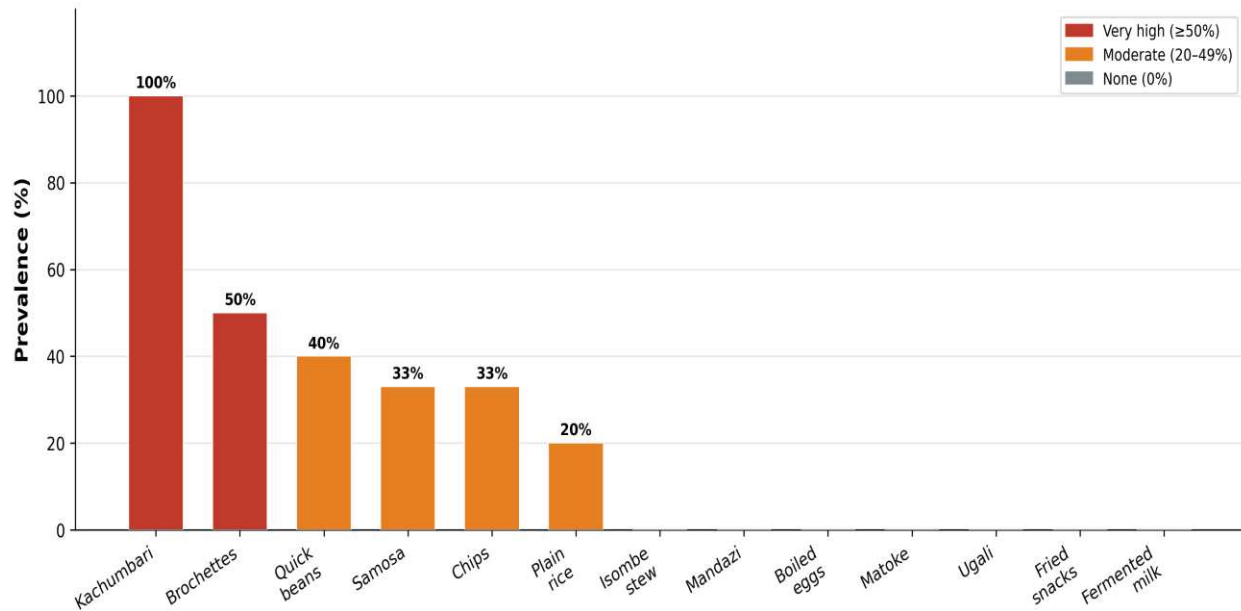


Fig. 3 Prevalence of *Escherichia coli* contamination (% positive samples) by food type ($n = 50$). Red: very high ($\geq 50\%$); orange: moderate (20–49%); green: zero detection. Kachumbari showed 100% positivity; several cooked foods indicated post-cooking recontamination.

Escherichia coli by food type, percent of samples that tested positive. Above bars, percentages are presented. Very high prevalence ($\geq 50\%$), moderate prevalence (20–49%), zero detection (0%). Kachumbari displayed universal contamination with *Escherichia coli*, whilst several cooked foods were positive, indicating post-cooking contamination.

3.1.4 *Staphylococcus aureus* contamination

Staphylococcus aureus, which can spread through contaminated hands, nasal passages, skin wounds, or respiratory droplets from food handlers, was detected in 28% of the samples (14 out of 50) (Fig. 4). Fermented milk showed a 100% positivity rate (2 out of 2), but this result should be interpreted cautiously due to the very small sample size ($n = 2$). This contamination is likely due to the milking, fermentation, or distribution processes. The low pH level of 4.1 was insufficient to eradicate *Staphylococcus aureus*, as it is highly resistant to acidic environments. Brochettes had a 67% positivity rate for *Staphylococcus aureus* (4 out of 6), likely due to manual handling during preparation without proper hygiene. Quick beans had a 60% prevalence (3 out of 5), Kachumbari 50% (2 out of 4), while Samosa and Isombe stew each had 33%, Chips

30%, and both Plain rice and Fried snacks had 20% (1 out of 5 each). Foods consumed hot after cooking, like Matoke and Mandazi, or those with minimal handling, such as Boiled eggs, showed lower or no detection rates. The presence of *Staphylococcus aureus* is directly linked to manual contact with food and inadequate hygiene practices. Fisher's exact test indicated a significant correlation between the extent of handling and the detection of *Staphylococcus aureus* ($p = 0.012$), with manually prepared foods showing higher contamination levels.

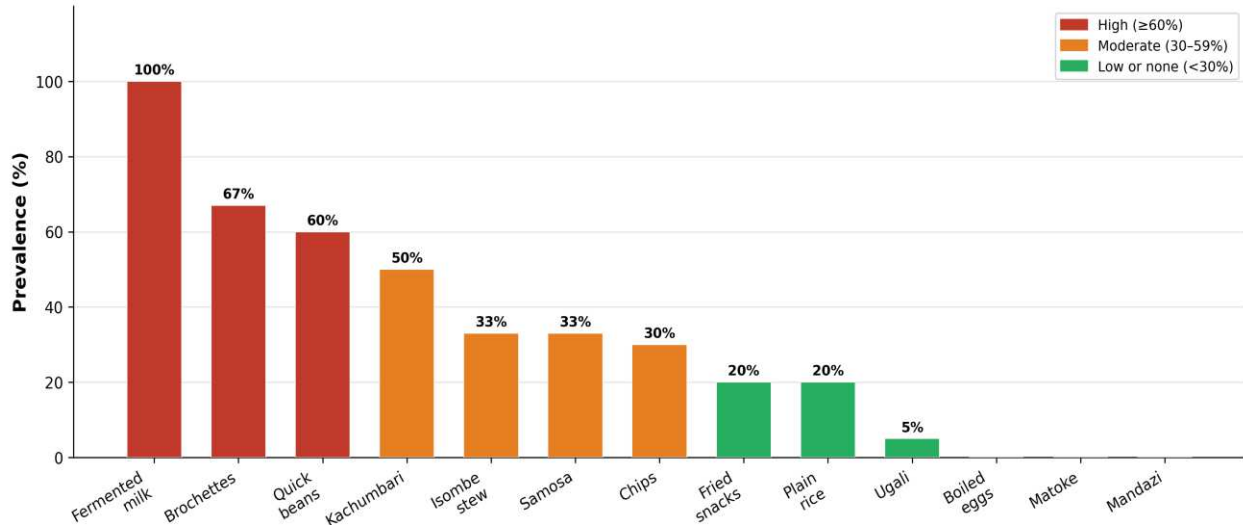


Fig. 4 Detection rate of *Staphylococcus aureus* (% positive samples) across food categories ($n = 50$). Red: high prevalence ($\geq 60\%$); orange: moderate (30–59%); green: low or zero ($< 30\%$). Fermented milk and brochettes had the highest contamination, attributable to extensive manual handling.

The percentage of positive samples in every food category is used to detect *Staphylococcus aureus*. High prevalence ($\geq 60\%$), moderate prevalence (30–59%), and low or zero prevalence ($< 30\%$) are represented by red bars, orange bars, and green bars, respectively. The highest contamination occurred in Fermented milk and brochettes, as there is a lot of manual handling during their production and serving.

3.1.5 *Salmonella* spp. contamination

Salmonella spp., a frequent culprit of bacterial foodborne illnesses, were identified in 36% of the samples (18 out of 50) through qualitative enrichment and selective plating (Fig. 5). The presence of *Salmonella* in ready-to-eat foods represents a significant health hazard, as this pathogen can be harmful even in small quantities (10–100 live cells), potentially leading to severe gastroenteritis characterized by bloody diarrhea, fever, and abdominal pain, especially in infants, the elderly, and those with weakened immune systems. *Salmonella* was found in Fermented milk (50%, 1/2), Quick beans (40%, 2/5), Fried snacks mix (40%, 2/5), Brochettes (33%, 2/6), Samosa (33%, 1/3), Isombe stew (33%, 1/3), Kachumbari (25%, 1/4), Chips (20%,

2/6), Plain rice (20%, 1/5), and Mandazi (20%, 1/5). However, no *Salmonella* was detected in Ugali, Boiled eggs, or Matoke, likely due to effective heat treatment, protective barriers, or immediate consumption. The widespread occurrence across different food types indicates systemic contamination issues, possibly due to cross-contamination from raw animal products, polluted water, insufficient cooking, recontamination via *Salmonella* ($\chi^2 = 19.3$, $df = 12$, $p = 0.033$). The detection in both raw and cooked foods highlights critical deficiencies in food safety practices from production to consumption. A comparative overview of pathogen detection prevalence (*Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp.) across all 13 food categories is presented in Fig. 9.

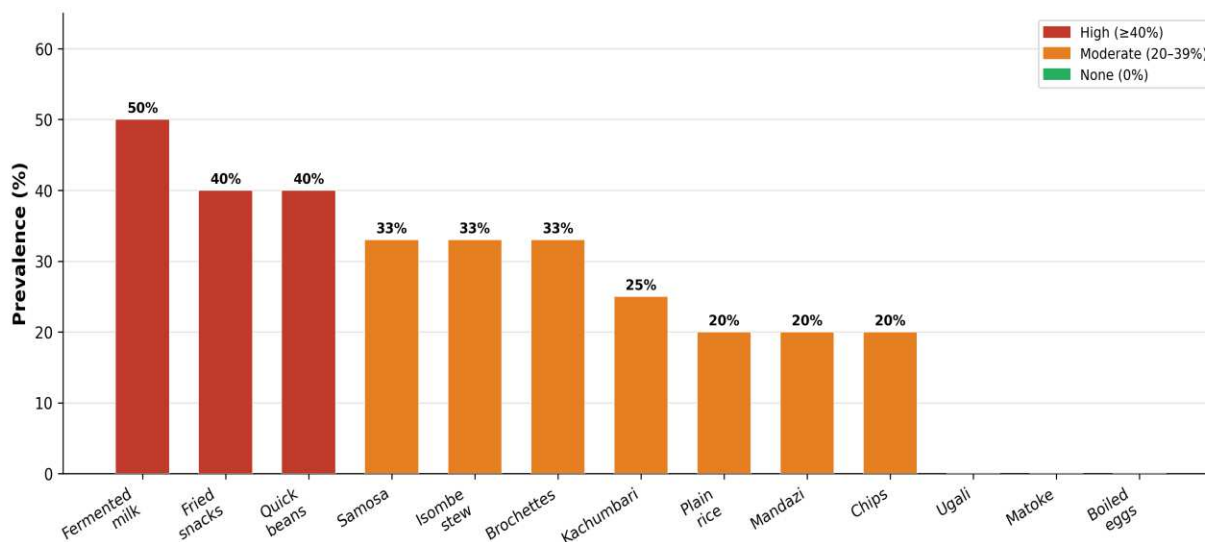


Fig. 5 Detection rate of *Salmonella* spp. (% positive samples) by food category ($n = 50$). Red: high ($\geq 40\%$); orange: moderate (20–39%); green: zero detection. Widespread contamination across food categories indicates systemic hygienic deficiencies and cross-contamination risks in the street food vending setting.

Detection of salmonella by food category. The high, moderate, and zero prevalence are indicated by red bars 40%), orange bars (20–39%), and green bars (0), respectively. The indications of extensive *Salmonella* contamination of various food categories indicate general hygienic deficiencies and cross-contamination concerns in the street food vending setting.

3.2 Temporal variations in microbial contamination

The quality of the microbiology of the ready-to-eat foods was also found to fluctuate with the time of sampling (morning: 07:00–09:00, midday: 12:00–13:00, evening: 18:30–19:00) depending on ambient temperatures, food holding time, and activity of the vendors (Fig. 6). Highest TPC ($6.4 \pm 1.9 \log_{10}$ CFU/g) and TCC ($5.2 \pm 2.1 \log_{10}$ CFU/g) were found in midday samples, while morning and evening samples

showed TPC ($5.1 \pm 2.0 \log_{10}$ CFU/g) and TCC ($3.8 \pm 2.2 \log_{10}$ CFU/g and $4.4 \pm 2.4 \log_{10}$ CFU/g). Kruskal-Wallis test indicated that microbial loads were significantly different in sampling times of TPC ($H = 7.2$, $df = 2$, $p = 0.028$) and TCC ($H = 6.8$, $df = 2$, $p = 0.033$). The Dunn test (with Bonferroni correction) revealed significant differences between the midday-evening and the morning-evening samples (adjusted $p < 0.05$). Large midday counts were due to maximum ambient temperatures ($25\text{--}31^\circ\text{C}$), long food holding periods (5–8 hours), and maximum vendor activity. The prevalence of pathogens fluctuated over the day: *E. coli* was maximum in the midday (18%, 7/20), and in the morning (10%, 3/15), and evening (8%, 2/15). *S. aureus* was highest midday (35%, 7/20) compared to morning (20%, 3/15) and evening (27%, 4/15), while *Salmonella* remained relatively constant: midday 40% (8/20), morning 33% (5/15), evening 33% (5/15).

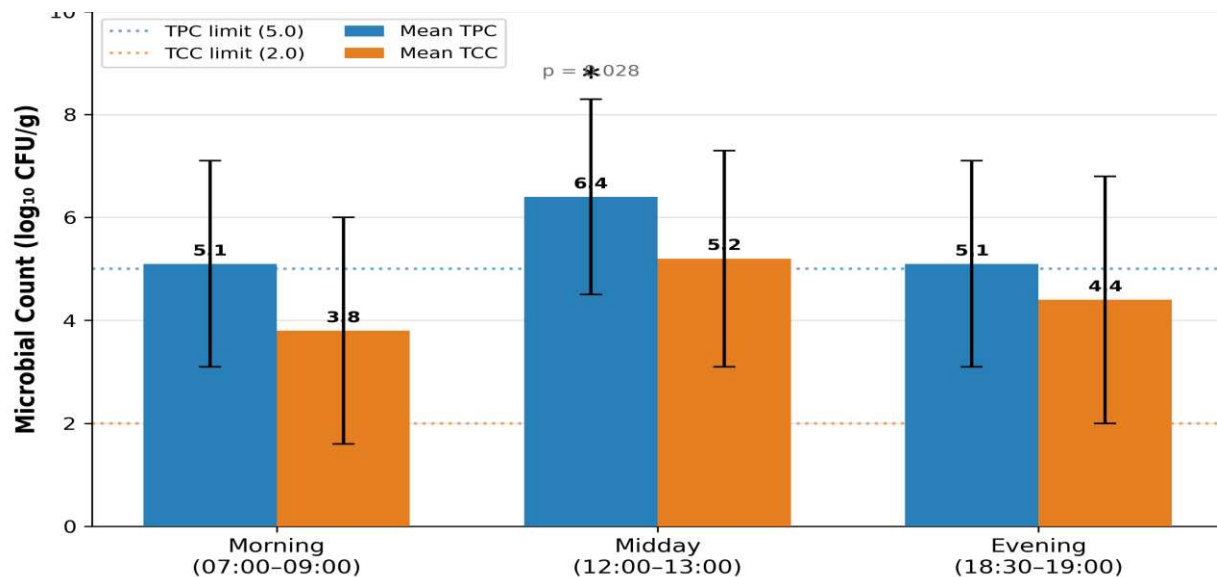


Fig. 6 Temporal variation in mean microbial loads (TPC and TCC, $\log_{10}$ CFU/g) by sampling period. Blue bars: mean TPC; orange bars: mean TCC. Error bars: standard error. Asterisk (*): statistically significant difference between midday and morning samples ($p = 0.028$; Kruskal–Wallis test). Midday samples showed the highest contamination, associated with peak ambient temperatures ($25\text{--}31^\circ\text{C}$) and extended food holding times (5–8 h).

Change in microbial load with time of sampling. Blue bars represent the mean TPC, and orange bars represent the mean TCC. The standard error of the mean is given by the error bars. An asterisk (asterisk) implies a statistically significant difference ($p = 0.028$) between midday and morning samples. Midday samples were most contaminated, and this was associated with the highest ambient temperatures and long holding periods.

3.3 Physicochemical parameters and their influence on microbial growth

Key intrinsic factors influencing microbial proliferation in food matrices include pH and water activity (aw). The mean pH was 6.3 ± 0.8 , with values spanning from 4.1 in fermented milk to 7.0 in boiled eggs. Most samples exhibited a nearly neutral pH (6.0-7.0), which is conducive to the growth of foodborne pathogens such as *Escherichia coli*, *Salmonella spp.*, and *Staphylococcus aureus*. Fermented milk had a pH of 4.1 ± 0.2 , attributed to lactic acid production, which inhibits *Escherichia coli* growth but permits *Staphylococcus aureus* growth due to its acid tolerance. Kachumbari's slightly acidic pH (5.8 ± 0.3) did not prevent pathogen growth because of its high water activity and absence of thermal processing. Water activity varied from 0.82 in Ugali to 0.99 in fermented milk, with an average of 0.93 ± 0.05 . Foods with $aw \geq 0.95$ included Kachumbari, fermented milk, quick beans, boiled eggs, and chips. The combination of high aw values, neutral pH, and ambient temperature provided ideal conditions for bacterial growth. Pearson correlation analysis revealed strong positive correlations between water activity and microbial counts (TPC: $r = 0.72$, TCC: $r = 0.68$, $p < 0.001$). Foods with lower aw exhibited reduced microbial counts, highlighting moisture's role as an antimicrobial preservation factor.

Antimicrobial susceptibility profiles of the recovered *Escherichia coli* and *Salmonella* isolates across the eight antimicrobial agents tested are summarised in Table 4 and Fig. 7.

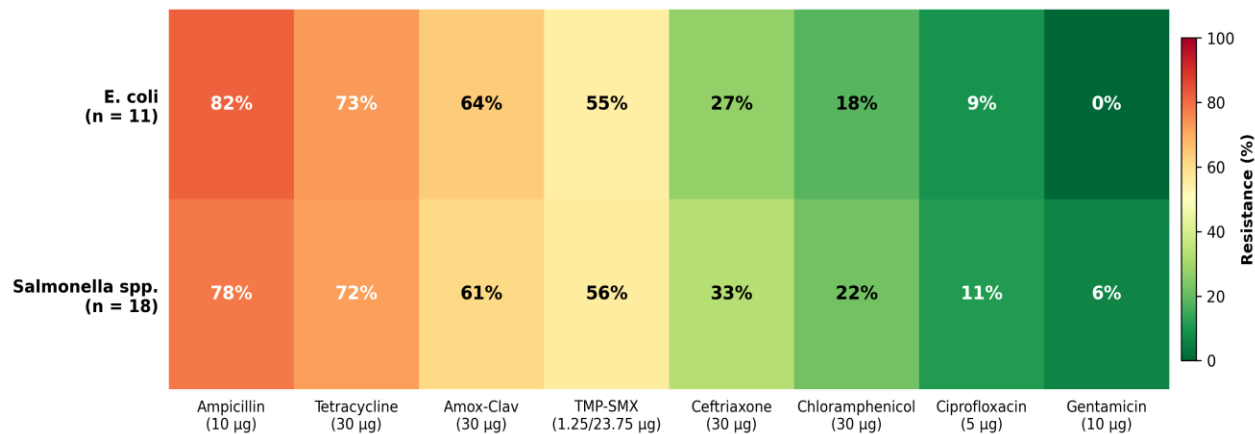


Fig. 7 Antimicrobial resistance heatmap showing percentage resistance of *Escherichia coli* ($n = 11$) and *Salmonella spp.* ($n = 18$) isolates to eight antimicrobial agents (Kirby–Bauer disk diffusion; CLSI M100). Colour gradient: green (0–20%), yellow (20–40%), orange (40–60%), red (>60%). MDR (resistance to ≥ 3 antibiotic classes) was observed in 64% of *E. coli* and 67% of *Salmonella* isolates.

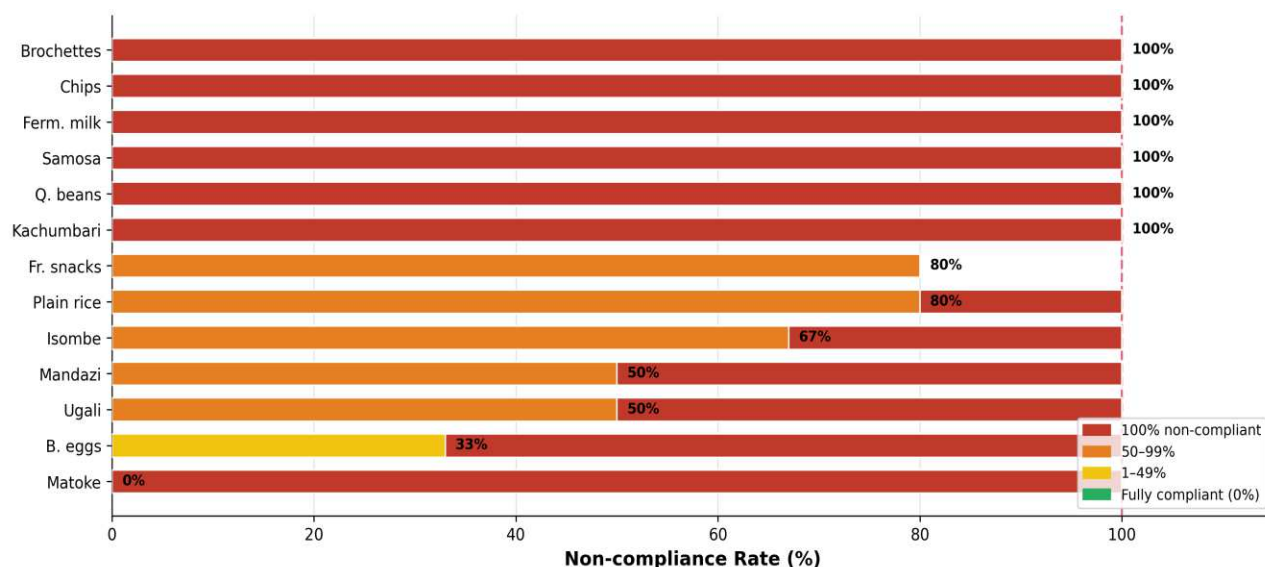


Fig. 8 Non-compliance rate (%) with EAC/FS 03:2021 microbiological standards by food category ($n = 50$). Six categories (Kachumbari, Brochettes, Fermented milk, Chips, Quick beans, Samosa) recorded 100% non-compliance with at least one safety threshold. Only Matoke achieved full regulatory compliance.

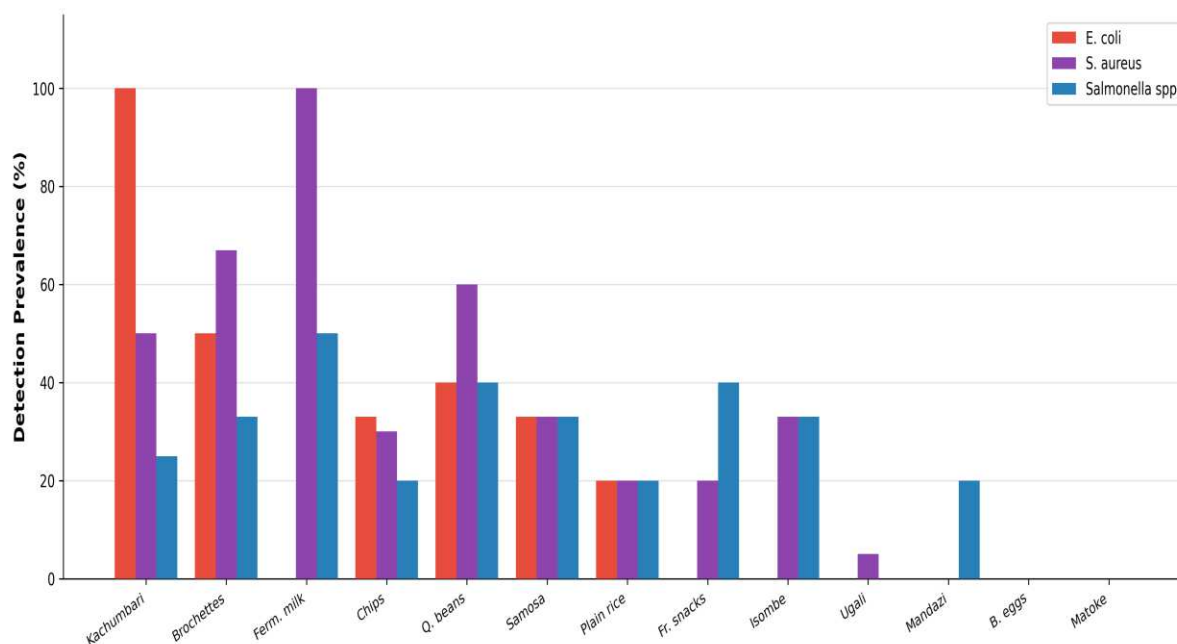


Fig. 9 Comparison of pathogen detection prevalence (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp.) across all 13 food categories ($n = 50$). Raw and protein-rich foods showed the highest concurrent pathogen burden, while heat-treated, low-moisture foods (Matoke, Ugali, Boiled eggs) showed the lowest detection rates.

Table 4. Antimicrobial resistance profiles of *Escherichia coli* and *Salmonella* spp. isolates recovered from ready-to-eat street foods

Antimicrobial agent (disk concentration)	<i>Escherichia coli</i> (n = 11)			<i>Salmonella</i> spp. (n = 18)	
	R n (%)	I n (%)	S n (%)	R n (%)	S n (%)
Ampicillin (10 µg)	9 (82)	1 (9)	1 (9)	14 (78)	4 (22)
Tetracycline (30 µg)	8 (73)	2 (18)	1 (9)	13 (72)	5 (28)
Amoxicillin–clavulanate (30 µg)	7 (64)	3 (27)	1 (9)	11 (61)	7 (39)
Trimethoprim–sulfamethoxazole (1.25/23.75 µg)	6 (55)	2 (18)	3 (27)	10 (56)	8 (44)
Ceftriaxone (30 µg)	3 (27)	4 (36)	4 (36)	6 (33)	12 (67)
Chloramphenicol (30 µg)	2 (18)	3 (27)	6 (55)	4 (22)	14 (78)
Ciprofloxacin (5 µg)	1 (9)	2 (18)	8 (73)	2 (11)	16 (89)
Gentamicin (10 µg)	0 (0)	1 (9)	10 (91)	1 (6)	17 (94)

n, isolates, *Escherichia coli* (n = 11 isolates in 6 *Escherichia coli*-positive samples); *Salmonella* (n = 18 isolates in 18 *Salmonella*-positive samples). The Kirby-Bauer disk diffusion method of susceptibility to antimicrobials was used based on CLSI M100 guidelines. Multidrug resistance (MDR, indicating resistance to ≥ 3 classes of antimicrobials): *Escherichia coli* 64% (7/11), *Salmonella* 67% (12/18). Red means extremely high rates of resistance (>60%). Partially effective is the intermediate susceptibility; clinical results can be different.

3.4 Regulatory non-compliance with EAC/FS 03:2021 microbiological standards

The microbiological parameters were measured against East African Community Food Safety Standard EAC/FS 03:2021 criteria for ready-to-eat foods. Non-compliance with at least one criterion was found in 72 percent of samples (36/50), indicating poor compliance among street food vendors. The non-compliance rate by food category is shown in Fig. 8. Six food categories (Kachumbari, Brochettes, Fermented milk, Chips, Quick beans and Samosa) showed 100% non-compliance, with samples exceeding multiple safety thresholds. These high-risk categories had plate counts exceeding the 5.0 log CFU/g limit in all samples. Total coliform counts showed 50-100% non-compliance across food types, with six categories recording 100% non-compliance. *Escherichia coli* was highest in Kachumbari (100%), Brochettes (50%), Quick beans (40%), and Chips/Samosa (33%). *S. aureus* was most detected in Fermented milk (100%), Brochettes (67%), Quick beans (60%), and Kachumbari (50%). *Salmonella* was highest in Fermented milk (50%), Quick beans and Fried snacks mix (40%), and Brochettes, Samosa, and Isombe stew (33%). Only Matoke showed complete compliance, while boiled eggs (33%), Mandazi (50%), and Ugali (50%) showed concerning but acceptable compliance levels. This systematic non-compliance indicates food safety

management failures at vendor level, requiring regulatory action, infrastructure improvements, and targeted safety training programs.

4. Discussion

4.1 Key Findings

The microbiological examination of ready-to-eat foods in Nyamirambo-Biryogo, Kigali, revealed significant contamination that poses a risk to public health. Seventy-two percent (72%) of the samples surpassed the EAC/FS 03:2021 safety limits, with average plate counts ranging from 6.8 to 7.4 log CFU/g, exceeding the regulatory cap of 5.0 log CFU/g. Pathogenic bacteria were detected, with *E. coli* present in 12% of samples, *Staphylococcus aureus* in 28%, and *Salmonella spp.* in 36% of Kachumbari samples. Antimicrobial testing indicated that 64% of *Escherichia coli* and 67% of *Salmonella* isolates exhibited multidrug resistance, showing high resistance to ampicillin (78-82%) and tetracycline (72-77%). The samples had higher microbial loads at midday compared to the morning ($p = 0.028$), linked to temperatures between 25-31°C and prolonged holding times. Bacterial proliferation was facilitated by a neutral pH (average 6.3) and high water activity (average 0.93, with 46% of samples exceeding 0.95), which correlated with total plate count ($r = 0.72$, $p < 0.001$) and coliform count ($r = 0.68$, $p < 0.001$).

4.2 Comparison with the existing previous studies in sub-Saharan Africa and beyond

Our findings are consistent with microbiological research on street foods in various urban areas. Similar levels of total plate counts and pathogen presence were observed in Nairobi, Addis Ababa, and Lagos [26], [27]. In Nairobi, [28], [29] identified *E. coli* in 15% and *Salmonella* in 28% of chicken samples, which is comparable to our findings of 12% and 36%, respectively. Our detection rate of *Staphylococcus aureus* was lower than the 45% reported in Dhaka street foods. The presence of *Escherichia coli* in Kachumbari (4 out of 4 (100%) samples) aligns with tests on raw vegetables in East Africa. [30], [31] reported that 89 % of raw vegetables from street vendors exceeded coliform limits, similar to our results. This indicates contamination of produce through irrigation, soil contact, and handling. Simply washing with water offers limited microbial reduction without the use of sanitizing agents. Our rates of antimicrobial resistance surpass regional reports, with ampicillin resistance at 82% compared to 40-60% in studies from Kenya and Tanzania between 2015 and 2018. Tetracycline resistance has risen to 73% from a previous range of 50-65%. This increase reflects growing resistance in low-income countries due to unregulated antibiotic access and inadequate regulation. Particularly concerning is the resistance to critical antibiotics like ciprofloxacin (9-11%) and ceftriaxone (27-33%). As these are last-resort treatments, resistance to them suggests potential treatment failures and a higher risk of mortality in vulnerable populations [32], [33].

4.3 Pathways and agents of contamination

The contamination patterns in street foods from Nyamirambo-Biryogo are explained by interactions and reinforcing factors. The intrinsic physicochemical properties facilitated microbial proliferation and pathogen persistence. A water activity (a_w 0.95 in 46% of samples) and a near-neutral pH (6.0-7.0 in 77% of foods) supported bacterial growth. There was a significant correlation between water activity and both total plate count ($r = 0.72$, $p < 0.001$) and coliform count ($r = 0.68$, $p < 0.001$), indicating that moisture content is a determinant of bacterial load. Fermented milk, with a pH of 4.1, inhibited pathogens through antimicrobial properties, although *Staphylococcus aureus* managed to survive in low pH conditions. Foods with lower water activity, such as Ugali (a_w 0.82) and Mandazi (a_w 0.88), exhibited reduced microbial counts, as less moisture aids in preservation without refrigeration. Contamination was higher in samples taken at midday (mean TPC 6.4 vs. 5.1 log CFU/g in the morning, $p = 0.028$) at temperatures between 25-31°C. Food prepared early in the morning without refrigeration accumulates bacteria rapidly, with pathogen generation times ranging from 20-60 minutes at 25-37°C. Poor hand hygiene resulted in *Staphylococcus aureus* contamination, most prevalent in foods handled manually (Fermented milk: 100%, Brochettes: 67%, Quick beans: 60%). *Staphylococcus aureus*, which colonizes human nasal passages, can be transferred to the hands [34], [35]. Insufficient handwashing practices contribute to the spread of *S. aureus*. The 36% prevalence of *Salmonella* suggests cross-contamination through shared utensils and environmental factors. *Salmonella* is found in raw poultry (20-60%), meat (10-30%), and eggs (1-10%), and can persist on contaminated surfaces [36], [37]. The presence of *Salmonella* in cooked foods indicates contamination after cooking, as cooking to 74°C eliminates it.

4.4 Public health implications and antimicrobial resistance threats

The recorded microbial contamination and pathogen prevalence have significant implications for public health in Nyamirambo-Biryogo and Kigali. Ready-to-eat street foods are a primary food source for low-income urban dwellers, with consumption up to 30% of meals in similar East African settlements [18], [38]. This consumption is higher in Nyamirambo-Biryogo due to limited kitchen facilities and constrained food budgets. High pathogen prevalence increases foodborne illness risk. *Salmonellosis* has an incubation period of 6-72 hours, causing symptoms like bloody diarrhea, fever, and vomiting lasting 4-7 days. In 5-10% of cases, bacteremia develops, potentially leading to hospitalization. Pathogenic *Escherichia coli* causes gastroenteritis, and EHEC can produce Shiga toxins, causing hemolytic uremic syndrome. The *Escherichia coli* contamination in Kachumbari raises concerns as EHEC outbreaks are often linked to raw vegetables. *Staphylococcus aureus* causes rapid-onset food poisoning with symptoms resolving in 24-48 hours [29]. Antimicrobial resistance presents additional health risks. Resistant *Escherichia coli* and *Salmonella* can lead to treatment failures, extended illness, and higher mortality risk [39], [40], [41]. Transient colonization

of the gastrointestinal tract creates a reservoir of resistant bacteria transferable to household contacts or the environment. Horizontal gene transfer between resistant bacteria and gut microbiota occurs through conjugative plasmids containing multiple resistance genes [42], [43]. Resistance patterns indicate the livestock origin of resistant isolates. In animal farming, ampicillin, tetracycline, and sulfonamides are widely used as growth promoters with little veterinary control in Rwanda and East Africa. Animals exposed to antibiotics may carry resistant bacteria that contaminate food products during processing. Addressing antimicrobial resistance requires One Health interventions uniting human health, animal health, and environmental health sectors [44], [45]. Without action, street-vended foods will continue spreading resistance in urban communities.

4.5 Implications of intervention strategies and policy development

The reported contamination trends require multi-sector intervention strategies. Infrastructure development is crucial for safe street food vending, including clean water access, handwashing facilities, refrigerated storage, hot hold equipment maintaining foods at 60 °C, covered bins, drainage, and protected vending booths [9], [46]. These investments need coordination between municipal governments, utilities, stakeholders, and vendor associations. African cities show infrastructure improvements work best when vendors pay maintenance fees, and municipalities supervise. Sustaining these gains is nonetheless challenging in developing economies, where limited vendor financing, fragmented enforcement, and competing municipal priorities remain persistent barriers to embedding food safety practices [47]. Food safety training must link to mandatory vendor licensing. Evidence-based training on hygiene, contamination prevention, temperature control, sourcing, and antimicrobial stewardship improves outcomes. Training should be participatory, in Kinyarwanda, delivered by local trainers, with visual aids and refresher courses. Licensing requires recertification and enforcement. Graduated licensing based on competency provides improvement incentives, consistent with evidence that vendor behaviour and attitudes are themselves key drivers of whether food safety standards translate into practice on the ground[48]. For kachumbari and raw salads, consistent with evidence-based food safety practices, interventions should include triple washing with chlorinated water (100–200 ppm free chlorine), sourcing from certified vendors, using dedicated cutting boards, and offering cooked alternatives during high-risk periods. For protein foods like Brochettes and Quick beans, ensure proper cooking temperatures of 74 °C, limit holding times to 2 hours, maintain hot-holding at 60 °C, and enforce reheating policies. For dairy items, source from pasteurized suppliers and maintain cold chain distribution. Incorporating vendors into Rwanda's food safety surveillance enables accountability through sampling. Risk-based inspections targeting non-compliant vendors can improve practices [49], [50]. Consumer education through media and health workers should promote safe practices, hot foods, avoiding raw foods, and illness awareness. Informed consumers motivate vendors to improve alongside regulation. Surveys of consumer knowledge, attitudes, and practices around raw and undercooked food consumption elsewhere show that awareness alone does not always translate into safer purchasing choices, reinforcing the need to pair consumer education with vendor-level safeguards

4.6 Study strengths and limitations

The paper presents methodological advantages increasing reliability through ISO-standard microbiological methods in an accredited laboratory. Detective profiling and antimicrobial resistance testing provided actionable public health data on food safety threats. Temporal sampling revealed significant contamination patterns, including a midday peak ($p = 0.028$) correlating with temperature [51], [52]. Combined physicochemical and microbiological data enabled interpretation of contamination drivers, with moisture

showing high correlation ($r = 0.68-0.72$). The focus on populated informal settlements makes the policy relevant for targeted interventions. However, limitations warrant consideration. The purposive sampling in one urban area (Nyamirambo-Biryogo, Kigali) limited statistical transferability to areas with different infrastructure and practices. While results represent upper-bound contamination levels in high-risk settings, extrapolation should be done cautiously. Sampling from January 2026 to September 2026 may still have missed additional seasonal variations that can affect contamination through temperature, humidity, and vendor behavior. Contamination might vary in the warmest months (August-September) or coolest ones (June-July). Vendor hygiene was measured through microbiological results and observation, not structured interviews. While contamination patterns indicate specific issues - hand hygiene (*Staphylococcus aureus*), cross-contamination (*Salmonella*), temperature abuse - we cannot attribute practices without behavioral data. Future studies should include hygiene checklists and sampling at control points to establish causality. Lack of environmental sampling prevented attribution to specific sources. Testing water would determine contamination causes, while surface sampling would guide sanitation targeting [53], [54]. Molecular characterization of resistance mechanisms was not conducted due to resource limitations. Genome sequencing would identify resistance determinants and inform dissemination mechanisms. Sequencing to determine clonal relationships using MLST or PFGE would distinguish between common source contamination and independent events. Cost-effectiveness analysis was not described, yet it provides important policy guidance. While we can suggest improvements based on risks, prioritizing interventions requires comparing costs and benefits. Economic models may find optimal intervention packages [55], [56].

5 Conclusions

In Nyamirambo-Biryogo, Kigali, a significant portion of ready-to-eat foods is found to be heavily contaminated with microorganisms, with 72% of the samples (36 out of 50) exceeding the safety standards established by EAC/FS 03:2021. High-risk foods, including raw vegetable salads, grilled meats, unpasteurized dairy, and fried starches, surpass regulatory limits: total plate counts range from 6.8 to 7.4 $\log_{10}$ CFU/g, exceeding the permissible limit of 5.0 $\log_{10}$ CFU/g, while fecal contamination indicators show total coliform counts reaching 8.1 $\log_{10}$ CFU/g, also above the limit. A notable percentage of *Escherichia coli* (64% of isolates) and *Salmonella* (67%) exhibit multidrug resistance, with high resistance rates to antibiotics such as ampicillin (78–82%), tetracycline (72–73%), and amoxicillin-clavulanate (61–64%), underscoring a significant threat of antimicrobial resistance in the food supply chain. Factors contributing to this contamination include neutral pH with high water activity, inadequate temperature control during ambient storage, poor hand hygiene facilitating the spread of *Staphylococcus aureus*, and cross-contamination from raw animal products. Tackling these health risks requires multi-sectoral interventions across the food system. Essential actions involve developing infrastructure for clean water and sanitation, offering food safety education alongside vendor licensing, and strengthening regulatory surveillance. Street

food vending plays a vital role in nutrition and livelihoods in urban areas of Rwanda and sub-Saharan Africa, making its safety crucial for public health and economic development.

6. Recommendations and Future Research Direction

The research recommends aligning street-food regulations with EAC/FS 03:2021, covering microbiological standards, vendor licensing, and penalties for violations. Ready-to-eat foods should be integrated into Rwanda's food safety and AMR monitoring frameworks, with regular sampling for TPC, pathogens, and resistance profiles in Nyamirambo-Biryogo. Vendors must complete mandatory pre-licensing and periodic training on food hygiene, HACCP principles, and antimicrobial stewardship. Freshly prepared foods should be maintained at 60°C if not consumed immediately and discarded after 2-4 hours, while cold items should be stored at 5°C or produced in small batches. Routine lab work should include AMR testing of *Escherichia coli* and *Salmonella*, with results publicly reported. A One Health strategy is essential to address antibiotic resistance in livestock supplying local markets. Educational materials in Kinyarwanda should highlight the risks of raw salads, undercooked meat, and MDR organisms. Vendor associations in Nyamirambo-Biryogo should develop codes of practice and mentorship programs. Local stakeholders should engage in food-safety campaigns, promoting Nyamirambo as a safe food destination. Regular microbial surveys should assess contamination and AMR trends using EAC/FS 03:2021 standards, contributing to national surveillance systems.

Future research can address knowledge gaps by: (1) broadening current studies to encompass a wider geographic and temporal scope through multi-site sampling in urban and rural areas, across cities, and throughout all four seasons to identify variations and determine which patterns are generalizable or context-specific; (2) assessing and characterizing vendor behavior and attitudes using validated tools to link contamination to specific behaviors and inform targeted intervention strategies; (3) conducting comprehensive environmental sampling (hands, utensils, water, surfaces, flies) to understand contamination pathways. This evidence will guide evidence-based policymaking and resource allocation to sustainably improve street food safety in Rwanda and influence regional initiatives in East Africa and beyond.

List of abbreviations

Abbreviation	Full form
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
aw	Water activity
BPW	Buffered Peptone Water
BSL	Biosafety Level

CAT	Central Africa Time
CFU	Colony Forming Units
CLSI	Clinical and Laboratory Standards Institute
EAC	East African Community
ESBL	Extended-Spectrum Beta-Lactamase
HE	Hektoen Enteric agar
HSD	Honestly Significant Difference (Tukey)
ISO	International Organization for Standardization
MDR	Multidrug Resistance (resistance to ≥ 3 antibiotic classes)
PCA	Plate Count Agar
QA/QC	Quality Assurance / Quality Control
RNEC	Rwanda National Ethics Committee
RSB	Rwanda Standards Board
RTE	Ready-to-Eat
RVS	Rappaport–Vassiliadis Soya broth
SPSS	Statistical Package for the Social Sciences
TBX	Tryptone Bile X-glucuronide agar
TCC	Total Coliform Count
TPC	Total Plate Count
TSI	Triple Sugar Iron agar
VRBL	Violet Red Bile Lactose agar
XLD	Xylose Lysine Deoxycholate agar

Declarations

Ethics approval and consent to participate. This study received ethical approval from the Rwanda National Ethics Committee (RNEC), reference number 123/CAF/2026, approved on 23 January 2026. Written institutional permission to conduct environmental food sampling was obtained from the Nyarugenge District Office (reference: NYR/DIST/FOOD/120/2024). All analyses were performed at the Rwanda Standards Board microbiology laboratory under BSL-2 biosafety conditions. This study involved collecting food samples purchased from street vendors in a public marketplace; no human subjects, biological samples, or personal data were involved, so formal consent to participate was not required under the approved ethics protocol (123/CAF/2026).

Consent for publication. Not applicable.

Availability of data and materials. The dataset generated during the research is available upon reasonable request.

Competing interests. The authors have declared no conflict of interest regarding the study.

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Authors' contributions. Conceptualization: GF; Methodology: GF; Software (statistical analysis and data visualization tools): GF and JN; Validation: DO; Formal analysis: GF; Investigation: DO and RE; Resources: BM; Data curation: DO; Writing – original draft: GF; Writing – review and editing: GF, JCD, and RE; Visualization: DO; Supervision: JCD; Manuscript revision, grammatical correction, and citation review: EH and RE; Regulatory and policy review: All authors read and approved the final manuscript.

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