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MICROBIOLOGICAL QUALITY AND ANTIMICROBIAL RESISTANCE PROFILES OF STREET-VENDED AWARA AND WAINA IN KADUNA STATE, NIGERIA

Audu Haruna Jimoh¹, Godiya Gadzama Bitrus¹, Bello Mujidat² and JC Igwe¹

¹Faculty of Pharmaceutical Sciences, Kaduna State university

²National Biotechnology Research and Development Agency

For Correspondence: haruna.audu@kasu.edu.ng

ABSTRACT

Background: Street-vended awara (soybean curd) and waina (fermented rice cake) are widely consumed ready-to-eat (RTE) foods in Kaduna State, Nigeria, whose microbiological safety remains poorly characterized. Informal vending conditions and the escalating spread of antimicrobial resistance (AMR), including extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, pose significant One Health concerns.

Objective: To assess the bacteriological quality of street-vended awara and waina, compare microbial loads and species distribution between food types, evaluate temporal contamination patterns, and characterize antimicrobial resistance profiles including ESBL production.

Methods: Forty samples (awara, n = 20; waina, n = 20) were collected aseptically from 10 vendors across five markets at morning (07:00–09:00 h) and evening (16:00–18:00 h) time points. Total viable counts were determined by pour-plate on nutrient agar. *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *E. coli* were isolated on selective media and identified biochemically. Antimicrobial susceptibility testing was performed by Kirby–Bauer disc diffusion (amoxicillin, ciprofloxacin, gentamicin, ceftazidime); multidrug resistance (MDR) was defined as resistance to ≥ 3 antimicrobial classes. ESBL production was confirmed by double-disc synergy test. Data were analysed using Wilcoxon rank-sum and Pearson's chi-square tests in R (v4.3.3).

Results: All 40 samples exceeded the RTE safety threshold (1×10^5 CFU/g). Awara had significantly higher mean counts ($\sim 1.1 \times 10^7$ CFU/g) than waina ($\sim 6.5 \times 10^6$ CFU/g; $W = 321$, $p = 0.0007$). *S. aureus* (31%), *K. pneumoniae* (27.5%), *P. aeruginosa* (23%), and *E. coli* (18%) were recovered, with no significant between-food difference in species distribution ($\chi^2 = 1.03$, $p = 0.794$). Amoxicillin and gentamicin resistance exceeded 75% across isolates; ESBL producers were detected in 30–40% of *E. coli* and 40–50% of *K. pneumoniae*. Evening samples consistently showed higher microbial loads.

Conclusion: Both foods harbor MDR bacteria, including ESBL-producing Enterobacteriaceae, far exceeding safe RTE limits. Vendor hygiene training, safe water access, and integration of street food surveillance into Nigeria's national AMR action plan are urgently needed.

Keywords: Street food; antimicrobial resistance; ESBL; awara; waina

1.0 INTRODUCTION

In developing countries, ensuring food security remains a major challenge; however, ensuring food safety within available food systems

represents an equally critical and often more immediate public health concern (Raposo *et al.*, 2021). Food borne illnesses caused by microorganisms represent a significant public

health concern linked to street foods, contributing substantially to disease burden and mortality (Makinde *et al.*, 2023). Furthermore, more than many diseases are either caused by or transmitted through food, with significant health impacts in vulnerable populations such as children, pregnant women, the elderly, and immunocompromised individuals (WHO, 2022). Food pathogens contaminate food at different stages in a food production chain. This contamination can be from the raw material, water, or air as well as unsafe food storage and processing (Asfaw *et al.*, 2022; Garba *et al.*, 2023).

Awara is an unfermented soybean product (also known as soybean curd). It is a soft cheese-like food produced by curdling fresh hot soy milk with either a salt or an acid. Soya bean is a rich source of protein and fat as well as vitamins and minerals hence it sometimes serves as meat substitute. Awara has been used as meat and cheese substitute in rural and urban areas of North and South West Nigeria. Local processing of soy-cheese is usually done at the home level with many avenues for possible contamination. Waina (also known as rice cake) is a culturally significant staple food prepared from fermented rice batter and

commonly sold in local markets, roadside stalls, and other informal food vending settings. Despite its nutritional value and cultural importance, the safety of waina remains largely unregulated and understudied, thereby creating potential hotspots for the transmission of antimicrobial-resistant (AMR) pathogens.

Compounding the contamination burden is the escalating global crisis of antimicrobial resistance (AMR). The emergence and spread of multidrug-resistant (MDR) bacteria through the food chain is of particular concern in Nigeria, where antibiotic use remains largely unregulated and surveillance systems are underdeveloped (Murray *et al.*, 2022). Extended-spectrum β -lactamase (ESBL)-producing organisms are among the most clinically significant resistance phenotypes, conferring resistance to a broad range of β -lactam antibiotics, thereby complicating the treatment of community-acquired infections (Adzitey *et al.*, 2021; Founou *et al.*, 2021). While several studies have examined microbiological quality in Nigerian street foods, data specifically characterizing the contamination burden and resistance profiles of awara and waina in Kaduna State are

limited. Kaduna, as a major commercial and population centre in North-West Nigeria, offers a representative context for examining food safety risks associated with informal food vending. This study therefore aimed to: (i) determine the microbiological quality of street-vended awara and waina; (ii) compare microbial loads and bacterial species distribution between food types; and (iii) characterize the antimicrobial resistance profiles, including ESBL production, among isolated bacteria.

2.0 MATERIALS AND METHODS

Study Design and Sample Collection

A cross-sectional microbiological survey was conducted in Kaduna State, Nigeria. A total of 40 food samples comprising awara ($n = 20$) and waina ($n = 20$) were collected aseptically from 10 randomly selected street food vendors across major markets (Central Market, Ungwan Rimi, KASU Market, Barnawa, and Kargi). Verbal consent was obtained from vendors before samples were purchased. These samples were collected before vending commenced. To capture temporal variation, samples were obtained at two time points: morning (07:00–09:00 h) and evening (16:00–

18:00 h). Samples were collected in sterile polyethylene bags, transported on ice to the laboratory, and processed within 2 h of collection.

Sample Processing and Microbial Enumeration

Each sample (1g) was aseptically homogenized in 9 mL of sterile 0.1% peptone water to obtain a 10^{-1} dilution. Serial ten-fold dilutions (10^{-2} to 10^{-6}) were prepared as appropriate. Aliquots (1 mL) were plated in duplicate using the pour plate method on Nutrient Agar (Oxoid, UK) and incubated at 37 °C for 24–48 h. Total viable counts (TVC) were calculated from countable plates (30–300 colonies) and expressed as colony-forming units per gram (CFU/g).

Isolation and Identification of Bacteria

Distinct colonies were subcultured onto selective and differential media, including Mannitol Salt Agar (MSA), MacConkey Agar, Eosin Methylene Blue (EMB) agar, and Cetrimide agar, followed by incubation at 35–37 °C for 24 h. Presumptive identification was based on colony morphology and Gram staining.

Isolates were further identified using standard biochemical tests, including catalase, coagulase, oxidase, indole, methyl red, Voges–Proskauer, citrate utilization (IMViC), urease, motility, and triple sugar iron (TSI) tests, following established protocols and Bergey’s Manual of Systematic Bacteriology. Target organisms included *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton Agar (Oxoid, UK) in accordance with Clinical and Laboratory Standards Institute (CLSI, 2022) guidelines. Bacterial suspensions were standardized to 0.5 McFarland turbidity and inoculated uniformly onto agar plates. A limited panel of antibiotics representing commonly used therapeutic agents in the study setting was selected, including amoxicillin (25 µg) and ceftazidime (30 µg) (β-lactams), ciprofloxacin (5 µg) (fluoroquinolone), and gentamicin (10 µg) (aminoglycoside). These antibiotics were chosen based on their clinical relevance and availability for routine

susceptibility testing in resource-limited laboratory settings.

. Plates were incubated at 37 °C for 18–24 h, after which zones of inhibition were measured and interpreted as susceptible, intermediate, or resistant based on CLSI breakpoints. Isolates were classified as multi drug-resistant (MDR) if resistant to at least one agent in three or more antimicrobial classes.

Detection of Extended-Spectrum β-Lactamase (ESBL)

ESBL production was assessed among *Escherichia coli* and *Klebsiella pneumoniae* isolates showing reduced susceptibility to ceftazidime using the double-disc synergy test (DDST). Amoxicillin–clavulanate (20/10 µg) and ceftazidime (30 µg) discs were placed 20 mm apart (center-to-center) on Mueller–Hinton agar plates inoculated with standardized bacterial suspensions. Plates were incubated at 37 °C for 18–24 h. ESBL production was indicated by the presence of a characteristic enhancement (“keyhole effect”) of the inhibition zone of ceftazidime toward the amoxicillin–clavulanate disc.

Statistical Analysis

All statistical analyses were performed using R software (version 4.3.3). Descriptive statistics (mean, standard deviation, and range) were calculated for microbial counts and resistance profiles. Data normality was assessed using the Shapiro–Wilk test. As data were non-normally distributed, differences in TVC between awara and waina were evaluated using the Wilcoxon rank-sum test. Associations between food type and bacterial distribution were assessed using Pearson’s chi-square test. Statistical significance was set at $p < 0.05$.

Media and Reagents

All culture media and reagents (Oxoid Ltd., UK, and other standard suppliers) were prepared according to manufacturers’ instructions. Media and glassware were sterilized by autoclaving at 121 °C for 15 min prior to use.

3.0 RESULTS

3.1 Microbial Load

Total viable counts from all 40 samples exceeded recommended limits for ready-to-eat foods (acceptable limit: $< 10^5$ CFU/g). In awara, TVC ranged from 3.1×10^6 to 1.91×10^7 CFU/g (mean $\sim 1.1 \times 10^7$ CFU/g), while waina TVC ranged from 3.8×10^6 to 9.2×10^6 CFU/g (mean $\sim 6.5 \times 10^6$ CFU/g) (Table 1). Microbial loads were consistently higher in awara samples across all vendors and time points. The Wilcoxon rank-sum test revealed a statistically significant difference in microbial load between awara and waina ($W = 321$, $p = 0.0007474$), confirming that awara samples harbored significantly higher bacterial burdens. A progressive increase in TVC was observed in evening samples relative to morning samples for both food types (Figure 3). Detailed microbial count data are summarized in Table 1.

Table 1. Summary of total viable counts (TVC) for Awara and Waina samples

Food Type	Range (CFU/g)	Mean $\pm$ SD (CFU/g)	n
Awara	$3.1 \times 10^6 - 1.91 \times 10^7$	$\sim 1.1 \times 10^7 \pm 4.2 \times 10^6$	20
Waina	$3.8 \times 10^6 - 9.2 \times 10^6$	$\sim 6.5 \times 10^6 \pm 1.8 \times 10^6$	20
Wilcoxon W	321	$p = 0.0007474$	Significant

Wilcoxon rank-sum test; significance threshold $p < 0.05$.

3.2 Prevalence of Bacterial Isolates

A total of four bacterial species were isolated from both food types: *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli*. In awara, *S. aureus* was the predominant isolate (34%), followed by *K. pneumoniae* (25%), *P. aeruginosa* (23%), and *E. coli* (18%). In waina, *K. pneumoniae* was most prevalent (30%), followed by *S. aureus* (28%), with *P. aeruginosa* and *E. coli* each contributing 23% and 18%, respectively. The distribution of bacterial species is presented in Table 2. Pearson's chi-square analysis revealed no statistically significant association between food type and bacterial species distribution ($\chi^2 = 1.0302$, $df = 3$, $p = 0.7939$).

Table 2. Percentage prevalence of bacterial isolates in awara and waina.

Organism	Awara (%)	Waina (%)	Overall (%)
<i>Staphylococcus aureus</i>	34	28	31
<i>Klebsiella pneumoniae</i>	25	30	27.5
<i>Pseudomonas aeruginosa</i>	23	23	23
<i>Escherichia coli</i>	18	18	18
Chi-square result	$\chi^2 = 1.03$, $df = 3$	$p = 0.7939$	Not significant

Chi-square test; significance threshold $p < 0.05$.

3.3 Antibiotic Resistance Profiles

All isolates exhibited resistance to at least two antibiotic classes, with amoxicillin resistance rates ranging from 75% (*E. coli*) to 90% (*S. aureus*) across species. Resistance to gentamicin was notably high, particularly among Gram-negative isolates (77–88%), while moderate resistance levels were observed for ciprofloxacin (60–75%). Multiple antibiotic resistance was common among all isolates. Full antibiotic resistance data are presented in Table 3.

Table 3. Antibiotic resistance profiles (% resistance) of bacterial isolates, with ESBL status.

Organism	Amoxicillin (%R)	Ciprofloxacin (%R)	Gentamicin (%R)	ESBL+
<i>S. aureus</i>	85	~68	~82	N/A
<i>K. pneumoniae</i>	80	~72	~87	Yes (~40–50%)
<i>P. aeruginosa</i>	82	~75	~88	N/A
<i>E. coli</i>	75	~60	~77	Yes (~30–40%)

N/A: Not applicable. ESBL: extended-spectrum β -lactamase. Note: ceftazidime results were used for ESBL screening (see Methods); quantitative %R for ceftazidime per isolate not shown separately.

3.4 ESBL Production

Phenotypic screening using the double-disc synergy test (DDST) identified isolates of *Escherichia coli* and *Klebsiella pneumoniae* exhibiting characteristics consistent with extended-spectrum β -lactamase (ESBL) production. A synergy effect (enhanced inhibition zone toward amoxicillin–clavulanate) was observed in a proportion of isolates. Based on this phenotypic pattern, ESBL-producing isolates were estimated at 30–40% among *E. coli* and 40–50% among *K. pneumoniae*. No ESBL-associated phenotype was observed in *Staph. aureus* or *Pseudomonas aeruginosa* isolates.

3.5 Temporal Variation in Contamination

Microbial loads were consistently higher in samples collected in the evening compared to morning samples from the same vendor. Additionally, a proportional shift toward environmental Gram-negative organisms, particularly *P. aeruginosa* and *K. pneumoniae*, was observed in evening samples.

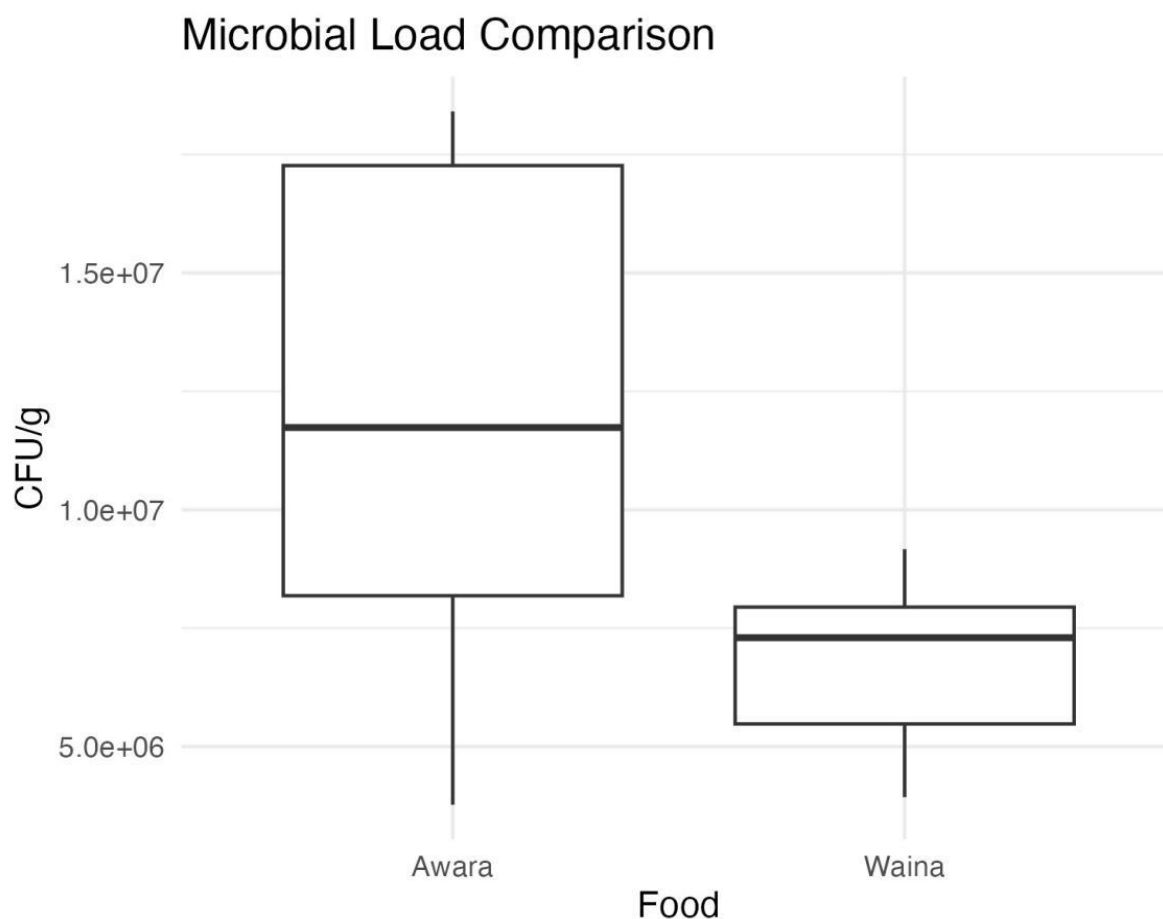


Figure 3. Comparison of microbial loads (CFU/g) between awara and waina. Box plots display median, interquartile range, and full data range. Awara samples showed significantly higher total viable counts (Wilcoxon rank-sum test: $W = 321$, $p < 0.001$).

4. DISCUSSION

This study provides an assessment of the microbiological quality and antimicrobial resistance burden of two widely consumed traditional street foods in Kaduna State, Nigeria. The principal findings are threefold: (i) both awara and waina harbor microbial loads substantially exceeding recommended thresholds for ready-to-eat (RTE) foods; (ii) awara carries a significantly higher bacterial burden than waina despite sharing a similar contamination ecosystem; and (iii) isolates from both food types exhibit high-level resistance to first-line antibiotics and include extended-spectrum β -lactamase (ESBL)-producing Enterobacteriaceae.

The significantly higher total viable count (TVC) in *awara* (mean $\sim 1.1 \times 10^7$ CFU/g) compared to *waina* (mean $\sim 6.5 \times 10^6$ CFU/g; $W = 321$, $p = 0.0007474$) likely reflects differences in product composition and post-processing handling. *Awara* is a fresh, moist soybean curd produced at ambient temperatures without the low-water-activity or

sustained heat treatment steps that characterize *waina*. The high moisture content and near-neutral pH of ready-to-eat foods are well-established determinants of microbial proliferation and reduced shelf stability in perishable foods (Makinde *et al.*, 2023). Similar findings have been reported for street-vended foods in Nigeria and other low- and middle-income settings, where total viable counts frequently exceed acceptable microbiological limits and commonly range between 10^6 and 10^8 CFU/g depending on handling and environmental conditions (Omorodion & Ogunekun, 2022; Profit *et al.*, 2021). In contrast, the detection of environmental organisms in *waina* samples even at early time points suggests that contamination may occur prior to or during processing, potentially via water sources, utensils, or preparation surfaces rather than being exclusively acquired during post-production handling (Garba *et al.*, 2023)..

Despite the quantitative difference in microbial burden, species-level distribution did not differ significantly

between the two food types suggesting that awara and waina are exposed to a common contamination organisms likely driven by shared environmental, hygiene, and handler-related risk factors, rather than product-related selective pressures. However, contamination dynamics varied over the vending period: early-stage contamination appears to be dominated by handler-linked organisms, particularly *Staphylococcus aureus*, whereas later time points show increased representation of environmentally persistent species such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. This pattern supports a transition from preparation-linked contamination to cumulative environmental exposure during vending.

The high prevalence of *Staphylococcus aureus* across both food types (31% overall) indicates frequent contamination from food handlers, as this organism is a well-recognized commensal of human skin and the nasopharynx. Its presence in ready-to-eat foods poses a risk of enterotoxin-mediated food poisoning, particularly under conditions of temperature abuse during vending that promote toxin production (Kadariya *et al.*,

2021). The detection of fecal indicator organisms like *Escherichia coli* and *Klebsiella pneumoniae* suggests poor personal hygiene, inadequate sanitation of equipment, or the use of contaminated water during food preparation, all of which are widely reported in informal food vending environments (Makinde *et al.*, 2023; Garba *et al.*, 2023). The predominance of *K. pneumoniae* in waina samples further supports environmental and fecal contamination pathways, with its persistence likely enhanced by its polysaccharide capsule (Paczosa and Mecsas (2016), which confers resistance to desiccation and other environmental stresses. The isolation of *Pseudomonas aeruginosa* in comparable proportions from both food types (23%) further reinforces the role of environmental contamination, given its its known prevalence in soil, water, and surface environments (Madigan *et al.*, 2015).

Amoxicillin resistance rates of 75–90% across all isolates are consistent with global and regional evidence showing widespread resistance to commonly used β -lactam antibiotics, particularly in settings with high levels of uncontrolled

antibiotic use (Laxminarayan *et al.*, 2013). The high level of gentamicin resistance observed (77–88%) is particularly concerning, as aminoglycosides are often reserved as second-line agents for Gram-negative infections and resistance reflects broader multidrug resistance selection pressures (WHO, 2022). The detection of extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* in street-vended foods is especially significant from a One Health perspective, as food products can serve as reservoirs and transmission pathways for clinically relevant antimicrobial resistance genes across human, animal, and environmental interfaces (Founou *et al.*, 2021).

The detection of ESBL-associated phenotypes in *Escherichia coli* and *Klebsiella pneumoniae* from ready-to-eat foods highlights a significant One Health concern, as antimicrobial resistance is increasingly recognized to circulate across interconnected human, animal, and environmental systems. Food products, particularly ready-to-eat foods, represent critical interfaces through which resistant

bacteria can be transmitted to humans. Studies have demonstrated that antimicrobial-resistant *E. coli* can be recovered from raw meats, ready-to-eat foods, and associated environments, indicating the movement of resistant strains across the food chain and reinforcing the One Health nature of antimicrobial resistance dissemination (Adzitey *et al.*, 2021).

In low- and middle-income settings, contamination of ready-to-eat foods is often linked to multiple sources, including contaminated raw materials, water, food contact surfaces, and human handling, all of which contribute to the persistence and spread of resistant foodborne pathogens (Asfaw *et al.*, 2022; Abalkhail, 2023). The presence of resistant Enterobacteriaceae in such foods is therefore not only a food safety issue but also a public health concern, as these organisms may serve as reservoirs of resistance determinants that can be transferred within the community.

Furthermore, the occurrence of antimicrobial-resistant foodborne pathogens in ready-to-eat foods has been consistently reported across different

regions, underscoring the global scale of the problem and the role of the food chain in the epidemiology of antimicrobial resistance (Mpundu *et al.*, 2021). These findings support the view that street-vended foods can act as both vehicles of infection and reservoirs of antimicrobial resistance, emphasizing the need for integrated One Health approaches that address food hygiene, environmental sanitation, and antimicrobial use across sectors.

The temporal increase in microbial load from morning to evening samples underscores the cumulative risk associated with prolonged ambient-temperature vending. This pattern is consistent with established food microbiology principles showing that time and temperature abuse significantly enhance bacterial growth in ready-to-eat foods (Makinde *et al.*, 2023). Moreover, the observed shift in species composition over time suggests that extended exposure not only increases microbial load but also influences the ecological profile of contaminants, favoring environmentally resilient organisms under suboptimal storage conditions (Garba *et al.*, 2023; Founou *et al.*, 2021).

Limitations

Several limitations of this study should be acknowledged. First, although the sample size was sufficient for detecting overall differences and conducting chi-square analyses, it limits the precision of species-specific resistance estimates. Second, the study relied on conventional culture and biochemical identification methods; incorporation of molecular techniques would provide greater resolution and mechanistic insight. Additionally, the absence of direct observational or quantitative assessment of vendor hygiene practices restricts the ability to attribute contamination to specific behavioral or environmental sources. The study also focused on a limited number of bacterial taxa; other important food-borne pathogens and fungal contaminants were not investigated. Finally, the cross-sectional design precludes causal inference regarding contamination pathways and temporal dynamics beyond the sampling window.

5.0 CONCLUSION

This study demonstrates that street-vended awara and waina in Kaduna State

harbor bacterial loads substantially above safe limits for ready-to-eat foods and serve as reservoirs of multidrug-resistant organisms, including ESBL-producing Enterobacteriaceae. While awara harbor's significantly higher microbial loads than waina, both food types share a comparable contamination ecosystem driven by common hygiene and environmental risk factors. The high amoxicillin and gentamicin resistance rates, combined with the detection of ESBL producers, underscore the food chain's potential role in the community spread of clinically relevant resistance phenotypes.

Targeted food safety interventions, including vendor hygiene training, improved access to potable water for food preparation, and enforcement of time-temperature vending regulations, are urgently needed. Routine surveillance of antimicrobial resistance in street foods should be integrated into national food safety and AMR action plans in Nigeria.

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Conflicts of Interest

The authors declare no conflicts of interest.

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