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ORIGINAL ARTICLE

PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF *SALMONELLA* SPP. ISOLATED FROM VEGETABLES IN FARMS AND MARKETS WITHIN ZARIA METROPOLIS, KADUNA STATE, NIGERIA

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

***Salmonella* is a significant zoonotic pathogen that causes diseases in humans and animals, often transmitted through contaminated foods, including vegetables. This study investigated the occurrence and antimicrobial resistance profile of *Salmonella* in vegetables obtained from farms and markets in Zaria metropolis. A total of 120 vegetable samples was processed following Clinical and Laboratory Standards Institute (CLSI) standards. An overall *Salmonella* prevalence of 15.8 % was observed from this study. Lettuce was the most contaminated vegetable (36.0 %), followed by onions (16.7 %), cabbage (12.0 %), cucumber (9.1 %), and carrots (4.2 %). Leafy vegetables had the highest contamination rate (23.1 %), compared to root (12.2 %) and fruit vegetables (5.3 %). Market samples showed a higher prevalence (18.3 %) than farm samples (13.3 %). Antimicrobial-resistance patterns of the positive isolates of *Salmonella* showed the highest percentage of resistance to erythromycin (100 %). Only 5.3 % of the positive isolates were resistant to ciprofloxacin, while none of the *Salmonella* isolates showed resistance to imipenem. Antibiotic susceptibility testing revealed that all isolates were resistant to at least one antibiotic, with 94.7 % exhibiting multidrug resistance. This study highlights the contamination of vegetables with *Salmonella* and its antimicrobial resistance patterns. It underscores the need for improved agricultural and retail practices to mitigate public health risks.**

Keywords: antimicrobial resistance; farm; market; Salmonella; vegetable; Zaria

INTRODUCTION

Fresh vegetables play a critical role in human nutrition, providing essential vitamins, minerals, and dietary fiber [1]. However, their consumption, particularly when raw or inadequately washed, poses significant public health risks due to their potential to act as vectors for pathogenic microorganisms linked to foodborne illnesses [2]. Epidemiological data have shown an increasing number of outbreaks associated with contaminated vegetables, often linked to the presence of coliforms, including *Escherichia coli* and *Salmonella*, which are indicators of fecal contamination [3]. These microorganisms are introduced through various contamination routes, such as the application of organic fertilizers, irrigation with contaminated water, direct contact with livestock, and poor post-harvest practices, including inadequate hygiene during handling and transportation [4]. *Salmonella* is one of the most prevalent causes of sporadic and outbreak-related gastroenteritis worldwide, with children under five, the elderly, and immunocompromised individuals being particularly vulnerable [5]. Mortality rates from *Salmonella* infections vary depending on serotype but can reach up to 21% in severe cases.

In Nigeria, the increasing consumption of ready-to-eat vegetables, driven by their established health and nutritional benefits, has not been matched by adequate attention to the potential microbiological hazards associated with these foods [6]. Studies have highlighted the contamination of raw vegetables with microorganisms, including *Salmonella*, due to poor hygiene practices and environmental factors [7]. Multidrug-resistant (MDR) *Salmonella* serotypes pose significant challenges to treatment efficacy and may lead to increased morbidity and mortality [8]. Factors such as limited food hygiene awareness among farmers, retailers, and consumers, as well as the growing prevalence of typhoid fever caused by *Salmonella* spp., further exacerbate the public health risks [9].

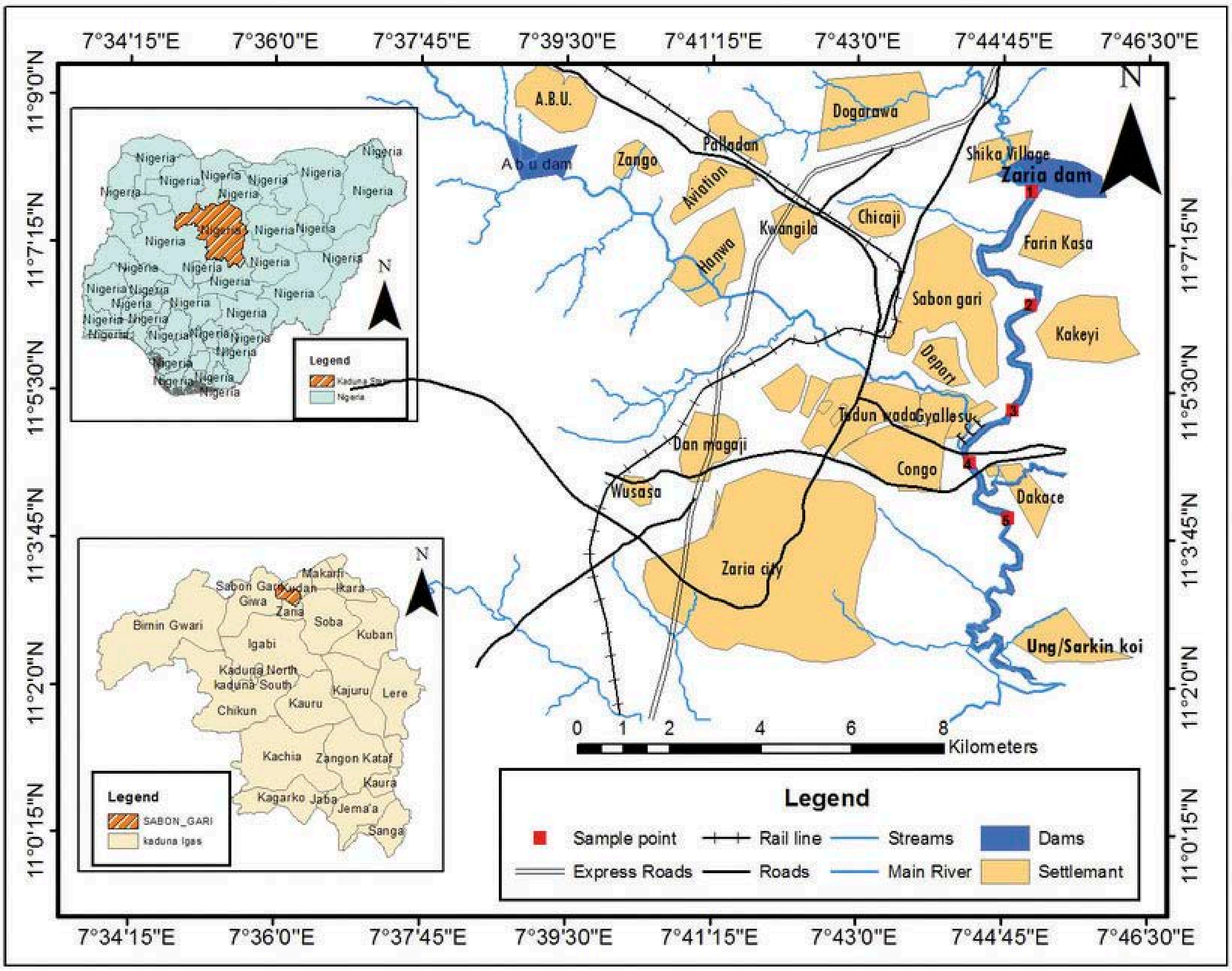

Fig. 1. Map of Zaria Metropolis (study area). Source: Satellite Image (2013)

This study aimed to determine the prevalence and antimicrobial susceptibility profile of *Salmonella* isolated from vegetables at the point of harvest and in markets within Zaria metropolis, Kaduna State, Nigeria. The findings will contribute to understanding contamination trends and inform public health interventions to mitigate the risks associated with vegetable consumption.

MATERIALS AND METHODS

Study area

The study was conducted in Zaria Local Government Area, located in Kaduna State in the northern region of Nigeria (Fig. 1). Agriculture is the primary occupation of the inhabitants, with a wide variety of vegetables grown and marketed within the area [10].

Study design

A cross-sectional study was conducted in July 2023 to investigate vegetable samples from farms and retail points in Zaria and Sabon-Gari local government areas, Kaduna State, Nigeria. Samples were collected based on availability, with prior consent obtained from participating farmers and retailers. A total of 120 vegetable samples—comprising carrot, cucumber, cabbage, onion, and lettuce—were systematically collected. Of these, 60 samples were obtained from five selected farms, while the remaining 60 were sourced from five selected retail markets within the study area.

Sample collection

Approximately 200 grams of each vegetable sample were collected in clean, sterile bags to prevent contamination and were appropriately labeled. The samples were stored and transported under chilled conditions (4 °C) using ice packs to the laboratory. All samples were processed within two hours of collection to ensure sample integrity.

Laboratory procedures

1) Pre-enrichment

Each vegetable sample was aseptically cut into smaller pieces using a sterilized knife, and 10 grams were weighed. The sample was transferred into a sterile stomacher bag containing 90 mL of peptone water, mixed for 1–2 minutes, and incubated at 37 °C for 24 hours to facilitate pre-enrichment.

2) Enrichment

For selective enrichment, Rappaport Vassiliadis medium was added to pre-enriched samples at a 9:1 ratio. The mixtures were appropriately labeled and incubated at 37 °C for 24 hours to promote the growth of *Salmonella* species.

3) Selective Plating

Using a sterile Pasteur loop, inoculated Rappaport Vassiliadis samples were streaked onto Bismuth Sulfite Agar (BSA) plates. The plates were labeled and incubated at 37 °C for 24 hours. Colony morphology was evaluated, with suspected *Salmonella* colonies appearing as black or brown colonies. These isolates were stored on nutrient agar slants in sterile sample bottles, incubated at 37 °C for 24 hours, and subsequently refrigerated for further analysis.

4) Biochemical Tests

Suspected *Salmonella* isolates were subjected to standard biochemical tests for confirmation. These tests included: Indole Production: Using SIM medium, Hydrogen Sulfide (H_2S) Production and Motility: Using SIM medium, Citrate Utilization: Using Simmons Citrate medium, Methyl Red (MR) and Voges-Proskauer (VP): Using MR-VP medium, Urease Production: Using urea broth. Additionally, isolates were inoculated into Triple Sugar Iron (TSI) agar and incubated at 37 °C for 24 hours. Isolates with typical Salmonella biochemical profiles (e.g., indole-negative, MR-positive, VP-negative, citrate-positive, motile, and H_2S production) were considered positive and stored on nutrient agar slants at 4 °C for further analysis.

5) Sugar Fermentation Test

Andrade's peptone water was used as a pH indicator to test sugar fermentation. Sugars (sucrose, mannitol, glucose, lactose, and maltose) were prepared according to the manufacturer's specifications and combined with Andrade's peptone water. Positive fermentation, indicative of Salmonella spp., was confirmed by a pink coloration due to pH changes, with lactose being an exception as it is typically not fermented by *Salmonella* spp.

6) Antibiotic Susceptibility Testing

Antibiotic susceptibility was evaluated using standard methods. The antibiotics tested included tetracycline, gentamicin, imipenem, ciprofloxacin, trimethoprim/sulfamethoxazole, and erythromycin. Inoculated plates were incubated at 37°C for 24 hours, and zones of inhibition were measured according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Results were categorized as sensitive (S), intermediate (I), or resistant (R) based on CLSI standards.

Data analysis

The collected data were analyzed using SPSS software (version 26). Descriptive statistics, including frequencies and percentages, were employed to determine the prevalence of *Salmonella* in the sampled vegetables. Chi-square tests were conducted to evaluate associations, with statistical significance set at a p-value ≤ 0.05.

RESULTS

The overall prevalence of *Salmonella* spp. in 120 fresh vegetables collected from selected markets and farms within Sabon-Gari and Zaria Local Government Areas was 15.8 % (19/120). Higher contamination rates were observed in samples from Zaria Local Government Area (21.7 %) compared to Sabon-Gari Local Government Area (10.0 %). However, no significant association was found between *Salmonella* prevalence and the source of the sample ($p = 0.509$). Market samples exhibited a higher prevalence of contamination at 18.3 % (11/60) compared to farm samples, which had a prevalence of 13.3 % (8/60) (Table 1).

Table 2 details the prevalence within the four markets. In descending order, *Salmonella* prevalence was highest in Tudun-Wada (26.7 %), followed by Galma Market (20.0 %), Samaru Market (13.3 %) and Sabo Market (13.3 %). Similarly, Table 2 also shows that farms in Tudun-Wada had the highest prevalence (26.7 %), followed by Gyellesu (13.3 %), Bomo (6.7 %), and Shika Farm (6.7 %).

Salmonella contamination was most prevalent in leafy vegetables (lettuce and cabbage) at 23.1%, followed by root vegetables (carrot and onion) at 12.2 %, with the lowest prevalence observed in fruit vegetables (cucumber) at 5.3 %. A statistically significant association was observed between *Salmonella* prevalence and vegetable type ($p = 0.025$) (Table 3).

Antimicrobial-resistance patterns of the positive isolates of *Salmonella* in Table 4, showed the highest percentage of resistance to erythromycin (100 %), followed by tetracycline (94.7 %), gentamicin (73.7 %), and trimethoprim/sulfamethoxazole (57.9 %). Only 5.3 % of the positive isolates were resistant to ciprofloxacin, while none of the *Salmonella* isolates showed resistance to imipenem.

According to Table 5, 18 (94.7 %) of the isolates exhibited multidrug resistance during the antibiotic sensitivity test, thus resistant to three (3) different classes of antimicrobials. Only 1 (5.3 %) isolate was resistant to two different classes of antimicrobials (tetracycline and erythromycin).

DISCUSSION

According to the World Health Organization [11], the presence of Salmonella in foods such as vegetables is a public health concern, as these foods can act as potential vehicles for transmitting the organism to humans and animals. The findings from this study reveal an overall Salmonella prevalence of 15.8 % in vegetables. This prevalence is higher than those reported by Yang et al. [12] in China (3.4 %), Raufu et al. [13] in Maiduguri, Nigeria (6.3 %), and Nair et al. [14] (4 %). However, it is lower than the prevalence reported by Azimirad et al. [15] in Iran (19.44 %) and Maikaji et al. [16] in Zaria (50 %). Variations in prevalence among studies may be attributed to differences in geographic and environmental factors, seasonal sampling, hygiene practices, and isolation techniques employed by researchers.

A higher *Salmonella* prevalence was recorded in Zaria Local Government Area (21.7 %) compared to Sabon-Gari Local Government Area (10.0 %). This disparity could be linked to Zaria's larger population, estimated at approximately 601,300 in 2021 compared to 430,000 in Sabon-Gari, leading to greater contamination risks through human and animal activities such as open grazing and defecation near farms. Additionally, questionnaire data revealed that most farmers in Zaria rely on the Kubani River for irrigation. This river has been reported as highly contaminated, partly due to effluents from an abattoir, which may contribute to the higher prevalence of Salmonella in this area [17].

Salmonella contamination of vegetables can occur at multiple stages of production, including cultivation, farm

Table 1. Prevalence of *Salmonella* isolates on vegetables grown and sold within Zaria metropolis, Kaduna State, Nigeria

Variables	Categories	Number Examined	Number positive	Specific Rate (%)	χ2	P-Value
LGA	Sabon Gari	60	6	10.0	0.436	0.509
	Zaria	60	13	21.7		
	Total	**120**	**19**	**15.8**		
Source	Market	60	11	18.3	0.563	0.453
	Farm	60	8	13.3		
	Total	**120**	**19**	**15.8**		

Table 2. Prevalence of *Salmonella* isolates on vegetables in selected markets and farms within Zaria metropolis, Kaduna state, Nigeria

Variables	Categories	Number sampled	Number Positive	Specific rate (%)	χ2	P-Value
Market	Sabo	15	2	13.3	1.939	0.747
	Samaru	15	2	13.3		
	T/Wada	15	4	26.7		
	Galma	15	3	20.0		
	Total	**60**	**11**	**18.3**		
Farm	Shika	15	1	6.7	3.564	0.468
	Bomo	15	1	6.7		
	T/Wada	15	4	26.7		
	Gyellesu	15	2	13.3		
	Total	**60**	**8**	**13.3**		

Table 3. Prevalence of *Salmonella* in different classes and types of vegetables from selected farms and markets within Zaria metropolis, Kaduna state, Nigeria

Variables	Categories	Number Examined	Number Positive	Specific rate (%)	χ2	P-Value
Class of Vegetable	Fruit Vegetable	19	1	5.3	4.114	0.128
	Root Vegetable	49	6	12.2		
	Leafy Vegetable	52	12	23.1		
	Total	**120**	**19**	**15.8**		
Type of Vegetable	Cabbage	25	3	12.0	11.119	0.025
	Carrot	24	1	4.2		
	Lettuce	25	9	36.0		
	Cucumber	22	2	9.1		
	Onions	24	4	16.7		
	Total	**120**	**19**	**15.8**		

Table 4. Antibiotic susceptibility profile of positive isolates of *Salmonella* on vegetables in farms and markets from Zaria metropolis, Kaduna State, Nigeria

Antibiotics	Susceptible (%)	Intermediate (%)	Resistance (%)
Tetracycline	1 (5.3)	0 (0)	18 (94.7)
Gentamicin	3 (15.8)	2 (10.5)	14 (73.7)
Imipenem	19 (100)	0 (0)	0 (0)
Ciprofloxacin	17 (89.5)	1 (5.3)	1 (5.3)
Trimethoprim/sulfamethoxazole	7 (36.9)	1 (5.3)	11 (57.9)
Erythromycin	0 (0)	0 (0)	19 (100)

Table 5. Antimicrobial resistance patterns of positive isolates of *Salmonella* on vegetables in farms and markets from Zaria metropolis, Kaduna State, Nigeria

Number of Antibiotics	Resistance Pattern	Number of Isolates (%)
2	TET, E	1 (5.3)
3	TET, E, CN	5 (26.3)
3	E, CN, SXT	1 (5.3)
3	TET, E, SXT	4 (21.1)
4	TET, E, CN, SXT	6 (31.6)
4	TET, E, CN, Cip	2 (10.5)

TET = Tetracycline, E = Erythromicin, SXT = Trimethoprim/sulphamethoxazole, CN = Gentamicin, Cip = Ciprofloxacin practices, harvesting, transportation, and post-harvest handling [4]. Market samples in this study exhibited a higher contamination rate (18.3 %) compared to farm samples (13.3 %), indicating additional contamination during post-harvest processes. This may result from factors such as cross-contamination between mixed vegetables, unhygienic transportation methods, the use of contaminated water for washing, open display on tables or floors, poor storage practices, and contact with infected individuals or fomites.

Among the five types of vegetables sampled, lettuce showed the highest prevalence of contamination, followed by onion, cabbage, cucumber, and carrot. The high contamination in lettuce can be attributed to its proximity to the ground, where soil-borne bacteria may be introduced through manure fertilization, and its large surface area, which increases susceptibility to contamination [18, 19]. Inadequate post-harvest washing by retailers further exacerbates this issue [20]. Across vegetable classes, leafy vegetables were the most contaminated, followed by root and fruit vegetables, consistent with findings by Franz et al. [21].

Antimicrobial resistance among the isolates is a growing global concern. The isolates from this study exhibited varying degrees of resistance to tetracycline, erythromycin, trimethoprim/sulfamethoxazole, gentamicin, and, to a lesser extent, ciprofloxacin. However, all isolates demonstrated high susceptibility to imipenem. The resistance trend for tetracycline aligns with findings by Lerttworapreecha et al. [22], attributing high resistance to the widespread use of tetracycline in livestock, its affordability, broad-spectrum activity, and easy accessibility. Similar results were reported by Toe et al. [23], highlighting multidrug resistance among *Salmonella* isolates. The observed multidrug resistance could be linked to human, animal, and environmental contamination. Contributing factors include the indiscriminate use of antibiotics in livestock farming and the misuse of antibiotics in human medicine. These practices emphasize the need for stricter antibiotic stewardship to mitigate the emergence and spread of multidrug-resistant *Salmonella* strains.

CONCLUSIONS

The findings in this study are of veterinary and public health importance, and they include: firstly, the study revealed that 15.8 % of vegetables grown and sold in selected farms and markets across Sabon-Gari and Zaria Local Government Areas in Zaria, Kaduna State, Nigeria, were contaminated with *Salmonella*. Secondly, *Salmonella* contamination was higher in market samples compared to those from farms, and almost all *Salmonella* isolates (94.7 %) exhibited multidrug resistance, highlighting a significant public health concern for vegetable consumers. Lastly, the two most effective antibiotics observed in this study were imipenem (100 % susceptibility) and ciprofloxacin (89.4 %), underscoring the pressing need to address the growing issue of antibiotic resistance.

We therefore recommend that regulatory bodies establish and enforce microbiological standards for farmers and marketers to ensure proper handling of vegetables. Public education campaigns should promote the responsible use of antibiotics in both livestock management and human medicine to mitigate resistance. Lastly, consumers should be advised to thoroughly wash vegetables, particularly those intended to be consumed raw, to reduce the risk of contamination.

Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

Ethical Statement

Ethical approval was not necessary for this work.

Conflict of Interest

No conflict of interest.

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Generative AI Statement

Authors disclosed that no form of generative AI and AI-assisted technologies were used in the writing process.

Authors' Contributions

Caleb V. O., Esonu D. O. and Ahnad A. I. designed the research, Caleb V. O. and Ahnad A. I. carried it out while

Esonu D. O. analyzed the data. Caleb V. O., Esonu D. O. and Ahnad A. I. were involved in writing and proof reading of the manuscript.

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