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Risk Factors and Occurrence of *Salmonella enterica* in Ready-to-Eat Vegetable Salad Sold in Gombe Metropolis, Gombe State, Nigeria

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Abstract

The worldwide twelve-monthly typhoid fever manifestations range amid 16-20 million ailments, ensuing in roughly 600,000 human mortalities, particularly amongst low-income and middle-income nations. Numerous typhoid outbreaks had previously been linked to contaminated vegetables. In the Gombe metropolis of Gombe state, Nigeria, ready-to-eat (RTE) vegetables are hawked around and handled using unhygienic methods. Hence, this study aimed to investigate and bridge the knowledge gap regarding the bacteriological safety of these ready-to-eat vegetables and the risk factors for their contamination with Salmonella enterica. A total of 100 samples were sourced from various locations in the Gombe metropolis, homogenized and inoculated on nutrient agar, Salmonella-Shigella agar, and MacConkey agar. Identification was executed by utilizing standard procedures of Gram's staining and biochemical tests. Antibiotic sensitivity assessment was executed per CLSI guidelines, a questionnaire was utilized to acquire data for ascertaining the association amongst handling and contamination of the RTE vegetable salad, and this data was analyzed using a chi-square test. Findings revealed 36% of the samples were contaminated with Salmonella enterica, which were grey-white on nutrient agar, colorless colonies with black centers on Salmonella-Shigella agar, non-lactose fermenters on MacConkey agar, Gram-negative rod-shaped, motility and catalase positive, indole negative, urease negative, citrate negative, and KIA positive. Sensitivity showed the isolates were 100% sensitive to ofloxacin and chloramphenicol, 100% resistant to ceftazidime, cotrimoxazole, and ceftriaxone, but 95.4% were sensitive and 4.6% resistant to ampicillin. Data analysis revealed a significant association between contamination and some handling practices. The findings in this study have shown that the RTE vegetable salad is a source of public health hazard to the community, and also the information generated by this study can be used to create targeted health interventions against the RTE vegetable salad-transmitted typhoid in Gombe, Nigeria.

Keywords: Risk Factors, Salmonella, Ready-to-eat Vegetable, Gombe, Nigeria

INTRODUCTION

Foodborne ailments present a worldwide public health issue encompassing parts of developed and developing nations. The World Health Organization (WHO) projected that in industrialised nations, approximately 30 out of every 100 individuals in the populace agonize at some point over contagions originating from food-borne ailments every twelve months, while in developing nations, about 2 million human mortalities are approximated to occur every twelve months due to this contagion (Xu and Yang, 2022).

The bacteria collectively known as *Salmonella* spp are amongst the highly noteworthy food-communicated disease-causing microorganisms globally, chiefly communicated between *Homo sapiens* via consumption of contaminated consumable items. Worldwide, millions of ailments due to gastroenteritis and hundreds of thousands of human demises are credited to these *Salmonella* spp every twelve months, amongst which *Salmonella* typhi has been renowned as an imperative serovar amongst the species that elicit such food poisonings (Nurjayadi *et al.*, 2019), .on a worldwide measure had accounted for around 16-20 million ailments of typhoid fever and an estimated 600,000 mortalities yearly (Moxley, 2022).

Enteric fever, which is a food-communicated contagion elicited by *Salmonella* serovars typhi and Paratyphi, persists as one of the tropical ailments of public health importance in Africa because of the elevated endemicity and transmission frequencies it possesses, chiefly in sub-Saharan Africa that presents 7.2 million

contagions of typhoid fever every twelve months and incidence rate of 762 out of 100 000 person-years in comparison to Northern Africa that presents incidence rate of 557 out of 100 000 person-years. More current research revealed that virtually all areas of sub-Saharan Africa have started inclining favourably towards elevated incidence frequencies, particularly in Central and Western Africa. Symptomatically undifferentiated from paratyphoid fever, typhoid fever elicits higher morbidity and mortality in comparison with paratyphoid fever, with elevated perils among young children. Risk features encompass ingestion of contaminated water, food eating from cooked food retailers, and recent interaction with an infected person or a chronic carrier of the pathogen, among other factors (Adesegun *et al.*, 2020).

Numerous ailments resulting from typhoid fever outbreaks have additionally been linked to consuming contaminated vegetables cultivated utilizing contaminated soil, fertilizer, manure, and sewage harbouring the typhoid pathogen. Such elevations in contagions due to fruits- and vegetables-borne *Salmonella* typhi could be attributed to amplified ingestion of these fruits and vegetables (which are most probably contaminated) outdoors because many individuals exhaust extended times away from home. In Nigeria, for example, outdoor hawking of convenient ready-for-consuming cut-up fruits and shredded vegetables has lately gained more popularity, and the business of such is booming (Bhunia, 2018).

In the Gombe metropolis of Gombe state, Nigeria, such ready-to-eat vegetables are frequently peddled about and handled by means of unhygienic approaches. This is alleged to create an avenue for contamination of the vegetables with pathogenic bacteria, including *Salmonella* typhi. Henceforward, this study aimed to investigate this issue and to bridge the existing knowledge gap regarding the bacteriological safety of these ready-to-eat vegetables in the Gombe metropolis. The specific objectives of the study included isolating and identifying *Salmonella enterica* from the RTE vegetable salad samples, determining the antibiotic sensitivity of the isolates, and identifying the risk factors for contamination of the RTE vegetable salad with these bacteria.

MATERIALS AND METHODS

Study Area

The location ranges designated for this study within the Gombe metropolis covered Tunfure to Bogo (west to east of Gombe metropolis) and Pantami to Arawa (south to north of Gombe metropolis) because the RTE vegetable salad vendors are usually mobile.

Study Design

A cross-sectional study design (Wang and Cheng, 2020) was utilized to amass samples and questionnaire responses simultaneously to circumvent mix-ups and repetitions since the vendors are habitually itinerant.

Sample Size and Sample Collection

A total of 100 samples were amassed. This sample number was chosen for the reason that it is thought to represent the populace of these vendors in the study ranges since they are not registered; hence, it is problematic to recognize their precise number for the resolution of sample size, and also majority of statisticians believe the minimum sample size to get any meaningful result from is 100 (Piroska, 2022). The samples were collected in fresh polythene mini sacks as retailed by the vendors and transported to the microbiology laboratory of Gombe State University for processing, experimentation, and analysis.

Sample Homogenization

From distinct samples, 9g were weighed and placed in 100 mL of sterile distilled water and blended for 2 minutes until entirely homogenized by means of a thoroughly disinfected blender (Yao *et al.*, 2021).

Isolation and Sub-Culture

1mL from distinct homogenized samples were diluted in 9ml of sterile distilled water in different test tubes, then 0.1ml from each dilution was inoculated using a spread plating technique onto distinct nutrient agar plates and then incubated at 37°C for 24h. The presumptive *Salmonella enterica* colonies were then sub-cultured by streaking method onto *Salmonella-Shigella* (SS) agar using a sterile inoculating loop and incubated for 24 hours at 37°C. These presumptive *Salmonella enterica* colonies from *Salmonella-Shigella* (SS) agar were further sub-cultured on MacConkey agar (Gwaza, 2020).

Identification: Culture Characteristics, Gram's Staining, and Biochemical Tests

Culture physical characteristics, which encompassed size, color, texture, transparency, and lactose fermentation, were utilized for presumptive identification of *Salmonella enterica* after isolation (Wang, 2022). Subsequently, standard methods of Gram's staining and biochemical tests, which included citrate utilization test, Kligler iron agar (KIA) test, urease test, indole test, motility test, and catalase tests, were used for identification of *Salmonella enterica* (Cheesbrough, 2006; Jabin *et al.*, 2021).

Antibiotic Sensitivity Assay

The sensitivity of the identified isolates to Ofloxacin (OFX 5µg), Chloramphenicol (C 30µg), Ceftazidime (CAZ 30µg), Cotrimoxazole (CLT 10µg), Ceftriaxone (CRO 30µg), and ampicillin (AMP 10µg), were elucidated by utilizing the guidelines of the Clinical and Laboratory Standards Institute. Prior to that, 24 hours old sub-cultures were used for standardization of the inocula to 0.5 McFarland standard using direct colony suspension method (CLSI, 2021).

Risk Factors Determination

Informed consents were obtained, followed by synchronous collection of questionnaire responses during sampling from 257 respondents (100 from the RTE vegetable salad vendors and 157 from other vegetable vendors that also sell the RTE vegetable salad when opportune) using a questionnaire adopted and modified from the work of Iwu *et al*. (2017).

Data analysis

The associations between the risk factors and occurrences of the *Salmonella enterica* were determined by analyzing the questionnaire data using Chi-square test for association at 95% confidence limit and significance level $p \leq 0.05$ (McHugh, 2013). The data analysis was done using IBM SPSS v25 software (Hinton, 2014).

RESULTS

The results for isolation (Table 1) revealed that thirty-six (36) samples (36%) were positive for presumed *Salmonella enterica* growth.

Table 1: Results of Isolation

Sampling Locations	Positive Samples	Number of Positive Samples
Pantami (A)	A_1, A_2, A_5, A_8	4
Bolari (B)	B_2, B_4, B_{10}	3
Nassarawo (C)	C_1, C_2	2
Tashan Dukku(D)	D_1, D_2, D_3, D_8, D_{10}	5
Old Market (E)	E_3, E_5, E_6, E_9	4
Tunfure (F)	F_1, F_5, F_6, F_9	4
Arawa (G)	G_4, G_9, G_{10}	3
Tashan Bauchi(H)	H_3, H_4, H_7, H_9	4
Tudun Wada (I)	I_5, I_9	2
New market (J)	J_1, J_2, J_6, J_7, J_{10}	5

Culture Characteristics

On nutrient agar, presumed *Salmonella enterica* were grey-white, moist, translucent circular colonies. On SS agar, the suspected *Salmonella enterica* colonies were circular and transparent with black centers (Plate 1), while on MacConkey agar, these colonies appeared as colorless-to-yellowish colonies, which are called non-lactose fermenters (Plate 2).

Gram's Staining and Microscopy

Suspected *Salmonella enterica* retained the Safranin stain and appeared pink in color when viewed under the microscope using x40 objective lens. When viewed using x100 objective lens (Plate 3) the suspected *Salmonella enterica* appeared as gram-negative pink colored bacilli.

Plate 1: Culture of *Salmonella enterica* on SS agar

Plate 2: Culture of *Salmonella enterica* on McConkey agar

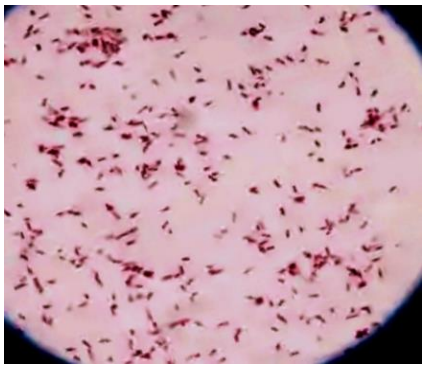

Plate 3: Suspected *Salmonella enterica* viewed under x100 objective lens

Biochemical Tests

The results for biochemical identification of the presumed *Salmonella enterica* are shown in Table 2. Also, some pictures of the biochemical test results are shown in Plate 4.

Table 2: Results of Biochemical Tests

Biochemical test	Outcome
Motility test	Positive
Catalase test	Positive
Indole test	Negative
Urease test	Negative
Citrate test	Negative
Kliegler Iron agar (KIA) test; hydrogen sulphide production	Positive
KIA test; lactose/dextrose fermentation	Negative
KIA test; slant reaction	Negative (red-red)
KIA test; butt reaction	Negative (alkaline-alkaline)

Plate 4: Some biochemical test results

Sensitivity Tests

The results of sensitivity tests (Table 3) revealed that these identified *Salmonella enterica* were completely resistant to ceftazidime, cotrimoxazole, and ceftriaxone. However, their susceptibility to ofloxacin and ampicillin displayed multiple outcomes, while they were completely sensitive to chloramphenicol. One of the sensitivity plates is shown in Plate 5.

Table 3: Results of Sensitivity Tests

Antibiotics	Concentrations	Numbers, percentages of isolates, and susceptibility patterns		
		Sensitive	Intermediate	Resistant
Ofloxacin	5µg	21 (58.33%)	15 (41.67%)	0 (0%)
Chloramphenicol	30µg	36 (100%)	0 (0%)	0 (0%)
Ceftazidime	30µg	0 (0%)	0 (0%)	36 (100%)
Cotrimoxazole	10µg	0 (0%)	0 (0%)	36 (100%)
Ceftriaxone	30µg	0 (0%)	0 (0%)	36 (100%)
Ampicillin	10µg	0 (0%)	13 (36.11%)	23 (63.89%)

Plate 5: Result of antibiotic sensitivity test

Risk Factors

The results for the Chi-square test (Table 4) revealed that the following variables were statistically significantly associated with occurrence of *Salmonella enterica* in the samples: not understanding food handling practice (Variable 1), no knowledge of food poisoning due to unhygienic RTE vegetable salad preparation (Variable 2), no knowledge regarding germs in the RTE vegetable salad (Variable 5), the RTE vegetable salad vendor not having refrigerator/stove/microwave (Variable 6), not washing hands with soap before preparing the RTE vegetable salad (Variable 7), not rinsing raw vegetables before processing (Variable 8), material used for cleaning the RTE vegetable salad containers (Variable 10), frequency of using new/fresh dish cloth/sponge/paper towel/sanitizer wipes (Variable 11), no knowledge of carrying harmful bacteria on hands/body (Variable 12), not knowing whether seller or buyer should be responsible for the RTE vegetable salad safety (Variable 15), how to identify contaminated RTE vegetable salad (Variable 16), implication of selling contaminated RTE vegetable salad (Variable 17), wearing uniforms when selling the RTE vegetable salad (Variable 18), method for serving the RTE vegetable salad (Variable 19), and method of packaging the RTE vegetable salad for customers (Variable 20).

Table 4: Results of Chi-Square Test for Risk Factors Determination

Variable ID (questionnaire items [see appendix 1])	FREQUENCY								Total Respondents	Pearson Chi-Square	Significance (*p*-value)
	Strongly agreed/ Very common/ Yes/Very Likely		Agreed/Somewhat common/No/ Somewhat likely		Partially agree/ Not very common/ Not sure/ Don't know		Not agreed/ I Don't know,				
	Yes	No	Yes	No	Yes	No	Yes	No			
1	15	117	15	71	0	24	0	15	257	7.942	.047
2	5	75	12	107	10	27	3	18	257	11.172	.011
3	18	121	12	63	0	24	0	19	257	7.264	.064
4	16	76	1	47	9	76	4	28	257	7.317	.062
5	0	93	12	58	11	47	7	29	257	19.422	.000
6	0	50	30	105	0	34	0	38	257	30.694	.000
7	6	93	18	44	5	74	1	16	257	23.886	.000
8	30	107	0	88	0	22	0	10	257	29.750	.000
9	20	109	10	75	0	29	0	14	257	7.520	.057
10	14	75	16	105	0	32	0	15	257	7.914	.048
11	1	64	10	64	19	81	0	18	257	14.304	.003
12	6	134	24	91	0	1	0	1	257	17.108	.001
13	8	73	22	150	0	3	0	1	257	.991	.804
14	14	135	16	84	0	3	0	5	257	3.622	.305
15	13	83	0	72	0	23	17	49	257	25.578	.000
16	8	67	10	117	3	31	9	12	257	21.926	.000
17	26	58	4	45	0	102	0	22	257	47.255	.000
18	8	71	14	96	8	25	0	35	257	9.984	.019
19	10	109	4	68	16	33	0	17	257	27.012	.000
20	7	48	22	50	0	29	1	100	257	39.970	.000

DISCUSSION

The results of isolation had shown that 36% of the RTE vegetable salad samples were contaminated with *Salmonella enterica*. This is thought to be due to the unhygienic methods associated with the RTE vegetable salad preparation (Abakari *et al.*, 2018), unsanitary environments where the RTE vegetable salad vendors are found (Abdul-Mutalib *et al.*, 2015), and unhygienic methods usually employed in serving the RTE vegetable salad products to consumers (King, 2020). These findings are in agreement with the works of Gómez-Aldapa *et al.* (2017), Gurler *et al.* (2015), Sant'Ana *et al.* (2011), and Taban *et al.* (2013), who all isolated *Salmonella* spp from RTE vegetables in Mexico, Turkey, Brazil, and Turkey, respectively.

The 36 isolates of *Salmonella enterica* were tested for their antibiotic susceptibility towards 6 antibiotics which included ampicillin, ceftazidime, ofloxacin, chloramphenicol, ceftriaxone, and cotrimoxazole using disk diffusion method, and the sensitivity pattern of an organism obtained for each of the antibiotic was interpreted as sensitive, intermediate or

resistant as per Clinical Laboratory Standards Institute (CLSI) guidelines.

Out of the 6 antibiotics tested against the *Salmonella enterica*, chloramphenicol and ofloxacin showed the highest antibacterial activity, while ceftriaxone, ceftazidime, and Cotrimoxazole were inefficient against the isolates.

The activity exerted by chloramphenicol and ofloxacin is thought to be due to their mechanisms of action, which are by inhibition of bacterial protein synthesis through binding with ribosomes of the bacteria (Abdollahi and Mostafalou, 2014) and by inhibition of bacterial DNA gyrase (Graham and Tripp, 2022), respectively. These findings are in agreement with the report of Nair *et al.* (2018a) whom had documented chloramphenicol-sensitive *Salmonella* spp isolated from a ready-to-eat food, and also with the work of Oluboyo *et al.* (2019), who had isolated ofloxacin-sensitive *Salmonella* spp in Ekiti State of Nigeria.

The resistance observed against ceftriaxone, ceftazidime, and ampicillin could be attributed to these *Salmonella enterica* producing CTX-M-type ESBLs (Shi *et al.*, 2021), which are a group of class A extended-spectrum ß-lactamases (ESBLs) that are rapidly spreading among Enterobacteriaceae worldwide (Rossolini *et al.*, 2008). These findings are in line with the reports of Nair *et al.* (2018b), who had documented ceftriaxone-resistant *Salmonella* Typhi in ready-to-eat vegetables in parts of the USA, of Yang *et al.* (2022), who had documented ceftazidime-resistant *Salmonella* Typhi in China, and that of Adzitey (2018) who had documented ampicillin-resistant *Salmonella* Typhi in Tamale metropolis of Ghana respectively.

The total resistance observed against Cotrimoxazole by all the isolates is because these *Salmonella enterica* were all sensitive to the fluoroquinolone ofloxacin (Karkey *et al.*, 2018). In the case of *Salmonella* spp, Cotrimoxazole can only be used to treat fluoroquinolone-resistant *Salmonella* spp, and this is because the isolate could be either a multidrug-resistant isolate or the circulating strain of H58 *Salmonella* Typhi (Britto *et al.*, 2018). These findings are in line with the report of Siddiqui *et al.* (2015), who had also reported cotrimoxazole-resistant *Salmonella* Typhi in ready-to-eat vegetables in Karachi- Pakistan.

The results of the risk factors determination had shown that some variables linked to knowledge about safe handling of food, attitude towards safe handling of food, and practice of safe food handling methods, were significantly associated with occurrences of *Salmonella enterica* in the RTE vegetable salad samples. This is not surprising because "not knowing safe food handling" and "not knowing what food poisoning is", as discovered in this study, will pre-dispose the vendors to poor hygiene (self and utensils), improper food (raw and processed) handling, and poor attitude towards seeking information to safeguard the health of their customers. In addition, the vendors' "lack of electronic food storage and reheating appliances" would encourage the proliferation and persistence of pathogens in this RTE salad (Xu *et al.*, 2024) which in turn puts the health of their customers in peril. Generally, these are thought to be due to the fact that lack of knowledge regarding safe food handling, improper food handling attitude, and lack of practicing safe food handling methods significantly contribute to contamination of food with pathogenic microorganisms (Auad *et al.*, 2019; Gyebi *et al.*, 2021). These findings are in agreement with reports of Ahmed *et al.* (2021) and Elobeid *et al.* (2019), who also isolated pathogenic microorganisms from food vended by individuals with poor knowledge, poor attitude, and deplorable practices regarding safe food production and handling in Pakistan and Qatar, respectively.

The strengths of this study can be attributed to the study design, which negated mix-up and repetitions in the sampling and data collection stages. In addition, the randomized approach to sample collection allowed for inferential statistics to be used in the data analysis. However, certain weaknesses which included refusal to participate by some vendors, may have limited the ability of the study to identify other significant risk factors.

Nonetheless, the findings of this study have identified a source of public health hazard to the population, and this information can be used by policy makers, health authorities, and other stakeholders to design and implement targeted health interventions against this particular threat to public health.

However, an important unanswered question that arose from this study is "where are the primary sources of the *Salmonella enterica* contamination?" and as such it is highly recommended that future research should focus on investigating this question.

CONCLUSION

The RTE vegetable salad sold in Gombe metropolis and the vendors involved are a source of public health risk to the community because of the *Salmonella enterica* contamination observed in this study and the vendor-associated factors linked to the contamination. It is believed that the findings from this study have shed more light on the bacteriological safety of the RTE vegetable salad and also will guide targeted health interventions for the

improvement of food safety and overall public health in the community.

Recommendation

Health education to vendors on personal hygiene and food safety should be done regularly, and also the Government should enforce regular monitoring and analysis of ready to eat foods.

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