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Cross-Sectional Study of *Salmonella* Species among Ready-To-Eat Fruit Salads

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Abstract

Salmonella enterica subspecies *enterica* serovar Typhi, commonly found in fruit salads, poses significant public health concerns due to its role in human infections and antibiotic resistance. Notably, approximately 80% of its resistance genes are plasmid-encoded, facilitating the spread of resistance and complicating treatment efforts. This cross-sectional study investigated the prevalence of enteric *Salmonella* species in ready-to-eat fruit salads sold in Uli community. A total of 100 samples were randomly collected from various hawkers and screened using standard microbiological techniques. The results revealed that 58.00% of the samples were positive for *Salmonella* serovar Typhi. Specifically, three strains were identified: *Salmonella enterica* subspecies *enterica* serovar Typhi strain CMCST (STCM), *Salmonella enterica* subspecies *enterica* serovar Typhi strain WG51146 (STWG), and *Salmonella enterica* subspecies *enterica* serovar Typhi strain R192829 (STR1). The occurrences of these strains were 31.03%, 24.14%, and 44.83%, respectively. Statistical analysis showed a significant presence of *Salmonella* serovar Typhi in the fruit salads. Notably, isolate STWG was the most predominant strain. The study's findings emphasize the importance of personal hygiene, community education, and proper fruit handling practices to control the transmission of *Salmonella* species. The high prevalence of *Salmonella* serovar Typhi in fruit salads poses a significant risk to public health, highlighting the need for effective interventions. This study's results can inform public health strategies to mitigate the risk of *Salmonella* infections in Uli community.

Keywords: *Salmonella Typhi, fruit salads, antibiotic resistance, public health, Uli community*

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1. Introduction

Salmonella is a rod-shaped, gram-negative bacterium and a facultative anaerobe belonging to the family Enterobacteriaceae. *Salmonella enterica* and *Salmonella bongori* belong to the genus *Salmonella*. Sofar, over 2600 serovars which belongs to *Salmonella enteric* have been described global, and several of these *Serovars* are able of causing different illness in both human and animals, while a

small number of variants of *Salmonella enteric* namely *Salmonella Gallinarum* SG) and *Salmonella* Pullorum (SP) are non-flagellated and non-motile, the greater part of members in the genus *Salmonella* are motile by peritrichous flagella. *Salmonella* Typhi is the aetiological agent of typhoid fever, while *Salmonella paratyphi* A, B and C cause paratyphoid fever. Since the clinical symptoms of paratyphoid fever are indistinguishable from typhoid fever, the term 'enteric fever' is used collectively for both fevers, and both *Salmonella* typhi and *Salmonella* paratyphi are referred to as typhoid *Salmonella* (Eng *et al.* 2015). For the two strains of typhoid *Salmonella*, humans are known to be the sole reservoir. The microorganisms are passed through the eating of infected food or water, with the waste of infected individuals. Prodomal symptoms of Enteric fever, such as headache, abdominal pain and diarrhoea (or constipation), are characterized by an incubation period of one week or more, followed by the onset of fever (Eng *et al*, 2015). Diarrhoea is more commonly observed in children, whereas patients with immunosuppression are more likely to display constipation (Eng et al, 2015). During the illness, enteric fever displays a specific fever pattern with an initial low-grade fever (>37.55^{0}C to 38.2^{0}C) which slowly develops to high-grade fever (>38.2^{0}C to 41.5^{0}C) in the second week. Fever can persist for a month or more, if the patient is left untreated (Patel *et al.*, 2018) Besides fever, infected patients, many also develop myalgia, bradycardia, hepatomegaly, splenomegaly, and rose acne on their chest and stomach (Kuvandik *et al*, 2017).

Salmonella infection owing to the economic burden of both modern and underdeveloped countries through the costs linked with surveillance, prevention and treatment of infection, remains a main public health concern worldwide (Eng, *et al*, 2015). Followed by bacteraemia and enteric fever, gastro enteritis is the most frequent symptom of *Salmonella* infection worldwide (Majonicz et al., 2010). *Salmonella* strains other than *Salmonella* typhi and *Salmonella aparatyphy* are stated as NTS, and are largely found in mammal reservoirs. NTS Infections are characterized by gastro enteritis or stomach flu, and inflammatory condition of the gastrointestinal tract which is accompanied by symptoms such as non-bloody diarrhea. Vomiting nausea, headache, abdominal cramps and myalgias. Hepatomegaly and splenomegaly symptoms are less commonly seen in patients infected with NTS (Eng *et al.*, 2015). Compared to typhoid infections, NTS infections have a shorter incubation period (6-12h), and the symptoms are usually self-limiting and last only for 10 days or less. (Cramp *et al.*, 2018). Gastrointestinal complications of NTS infections include cholecystitis, pancieatitis, and appendicitis (Acheson and Hohmann, 2001). Infants, young children, elderly people and immunocompromised patients are extremely prone to NTS infections and show more severe symptoms than normal individuals (Scallen *et al.*, 2016). *Salmonella* bacteraemia is a state in which the bacteria enter the bloodstream after invading the intestinal barrier. Nearly all the serotypes of *Salmonella* can cause bacteraemia, while *Salmonella* Dublin and *Salmonella* choleraesuis are two invasive strains that are highly associated with the manifestation of bacteremia (Eng *et al.*, 2015). Similar to enteric fever, high fever is the characteristic symptom of bacteraemia, but without the formation of rose spots as observed in patients with enteric fever. In harsh conditions, the immune response triggered by bacteraemia can lead to septic shock, with a high death rate.

The appearance of antimicrobial resistance in *Salmonella* strains is a severe health concern worldwide. (Eng *et al.*, 2015). In the early 1960s, the first incidence of *Salmonella* resistance to a single antibiotic, namely chloramphenicol, was reported (Eng *et al.*, 2015). Since then, the occurrence of separation of *Salmonella* strains with resistance towards one or more antimicrobial agents has improved in many countries, including the USA, the UK and Sandi Arabia (Eng *et al.*, 2015) Antimicrobial agents such as amplicillin, chloramphenicol and trimethoprimsulfamethoxazole are used as the traditional first line treatments for *Salmonella* infection. *Salmonella* spp resistant towards these agents are referred to as multidrug-resistant (MDR), with the emergence of resistance towards traditional antibiotics. Fluoroquinolones and extended-spectrum cephalosporins have been introduced as the antimicrobial agents of choice in treating MDR *Salmonella* typhi.

2. Materials and Methods

Sample collection, handling and transportation

A total of 100 samples, twenty samples from each location were used for this study**.** The samples used for this study were collected from different hawkers in Uli community. In each location, the sample was collected from top, middle and bottom.This sample was covered immediately and kept in a cooler containing ice block, and this transported to the laboratory for immediate analysis. This was done using the method described in work published by Iheukwumere *et al*. (2025e).

Isolation of organisms

One gram (1.0 g) of sample was aseptically transferred into a sterile test tube (Pyrex), then 3 ml of diluent (sterile normal saline) was added and then made up to 10 ml, and from this, ten-fold serial dilutions were made up to 10^{-3}. One milliliter of the diluted sample (10^{-3}) was plated on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing Deoxycholate agar medium (DCA/Biotech) using the pour plate method. All the plates in triplicate were incubated inverted at 37±2˚C for 24-48 h.

Characterization and identification of the isolates

The isolates were subcultured on nutrient agar (Biotech), incubated in an inverted position at 37±2˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions as described in the study published by Iheukwumere *et al*. (2018), Iheukwumere *et al*. (2025a), Iheukwumere *et al*. (2025b), biochemical reactions as described in the study published by Iheukwumere *et al*. (2020), Iheukwumere *et al*. (2025c) and molecular characterization as described in the study published by Gabriela *et al*. (2014) and Ekesiobi (2015).

Prevalence and Distribution of the Isolates in the Fruit Salad Samples

The number of each bacterial isolate in each sampling area was enumerated, and these were calculated as a percentage of the occurrences. The bacteria that appeared in each sample location were detected and recorded as described in the study

published by Iheukwumere *et al* (2021), and Abiodum *et al*. (2024b).

Statistical Analysis

The results of the data generated were expressed as mean, percentage and Table, Data were analyzed by two-way Analysis of Variance (ANOVA) to determine the significance of the main effects and interactions at 95 % confidence level. Pair wise comparison of mean was done by Student "t" test sa described in the study published by Iheukwumere *et al* (2018), Ekesiobi *et al*. (2017), Abiodum *et al*. (2024a), Abiodum *et al*. (2024c), Iheukwumere *et al*. (2025d).

3. Results

The study revealed that 58% of the samples were positive for *salmonella* species. The fruit salad samples drawn from shop c showed the highest occurrences of the test organisms whereas shop b recorded the lowest occurrences.

The study revealed that the isolates exhibited similar appearances on Deoxycholate citate agar and also similar morphological characteristics such as Gram reaction, cell morphology and nature

The biochemical characteristics of the isolates revealed that the isolates were catalase, hydrogen sulphide production and methyl red positive, and oxidase, citrate, indole, urease and voges prokarier`s negative as shown in table 3. The isolates differ in their variation in utilization of sugars. They were all glucose, maltose and tetric halose positive but differ in their abilities to utilize galactose, xylose, sorbitol, inositol and dulsitol.

The molecular acids_extracted from the isolates showed the ratio of their absorbances at wavelength of 260nm and 280nm using Nanodrop was at the range of 1.80 – 1.90,and this confirmed that the nucleic acids were DNA as shown in Table 4. The molecular identities of the isolates revealed that isolates x ,y and z were *Salmonella enterica* subspecies *Enterica* sewvar typhi srain cmcst (STCM), *Salmonella enterica* subspecies *enterica*_sewvar typhi strain R192829(STRI) and *salmonella enterica* subspecies *enterica* sewvar typhi strain WGS1146 (STWG)as shown in Table5.

The study also revealed that STWG should the highest occurrence in the studied stream samples whereas STRI recorded the least occurrence as shown in Table 6.

Table 1: Occurrences of the Bolates in the Sample

Stream	number drawn from the site (N)	P (%)	N (%)
A	20	12(60.00)	8(65.00)
B	20	7(35.00)	13(65.00)
C	20	16(80.00)	4(20.00)
D	20	9(45.00)	11(55.00)
E	20	14(70.00)	6(30.00)
Total	100	58(58.00)	42(42.00)

Table 2: Cultural and Morphological Characteristics of the Isolates

Parameters	**Isolate (x)**	**Isolate(y)**	**Isolate (z)**
Appearances	Colourless and dark centered on DCA	Colourless and dark centered on DCA	Colourless and dark centered on DCA
Edge	Entire	Entire	Entire
Elevation	Convex	Convex	Convex
Surface	Smooth	Smooth	Smooth
Gram reaction	-	-	-
Cell morphology	Rods	Rods	Rods
Endospore	-	-	-
Position of the Spore	-	-	-
Bulging	-	-	-
Motility	+	+	+

.**Table 3:** Biochemical Characteristics of the Isolates

Parameters	**x**	**y**	**z**
Catalase	+	+	+
Oxidase	_	_	_
Citrate	_	_	_
Indole	_	_	_
Urease	_	_	_
Methylred	+	+	+
Vogas prokarier	_	_	_
H2S	+	+	+
Glucose	+	+	+
Maltose	+	+	+
Galactose	_	+/-	+/-
Xylose	+	+/-	+/-
Sorbitol	+/-	+/-	+
Inositol	+	+/-	+
Dulsitol	_	+/-	_
Tetrahalose	+	+	+

Table 4: Nucleic Acids Extracted from the Isolates.

Isolate code	GCN (ng/rul)	280nm	260nm	260/280
X	102.40	1.6802	3.0580	1.82
Y	108.10	1.6940	3.0661	1.81
Z	120.20	1.7002	3.1284	1.84

Table 5: Molecular Identities of the Isolates.

Parameters	X	Y	Z
Max score	7239	13573	6593
Total score	7239	13573	6593
Query cover (%)	100	100	100
E-value	0.0	0.0	0.0
Identity (%)	100	100	100
Accession length	4861882	4812688	4813117
Accession number	cp053702	cp046429	cp040575
Description	*Salmonella enterica* subspecies *enterica* sewvar typhi strain CMCST (STCM)	*Salmonella enterica* subspecies *enterica* sewvar typhi strain R192829 (STR1)	*Salmonella enterica* subspecies *enterica* sewvar typhi strainWG51146 (STWG)

Table 6: Occurrences of the Bolates in the Samples

Isolates	Number	Percentage (%)
STCM	18	31.03
STRI	14	24.14
STWG	26	44.83
Total	58	100.00

4. Discussion

The presence of enteric bacteria in the studied samples could be traced from the handling, preparation and transportation conditions attributed to the salad samples. Similar findings were reported by many researchers (Immerseel *et al.*, 2014; Jones and Richardson, 2014; Alshawabkeh, 2016; Maciorowski *et al.*, 2017). Researchers had shown transportation of equipments can also harbour enteric bacteria and this contributes to the contamination of salad samples (Primm, 2018). Maciorowski *et al.* (2017) also stated that the high prevalence and high populations of enteric bacteria in salad samples can contribute to human food-borne illness through the salad-food-human chain. This shows that the preparation of fruit salads requires microbiological safety regulations to escape microbial contamination of the product. Similar deduction was drawn by different researchers (Iheukwumere *et al.*, 2018a; Iheukwumere *et al.*, 2018b; Kupryś-Caruk *et al.*, 2018).

The variation of enteric *Salmonella* from different samples of fruit salad studied could be attributed to the type of fruits used, personal hygiene and production location. Maciorowski *et al.*, (2017) reported that variation in microbial counts in different samples depend on the water activity, oxygen tension, pH and nutrient composition of the sample. Barakat, (2004) also reported that the vegetable protein sources, cereal grains and their by-products were among the factors that contributed to the variations in enteric bacterial counts in different samples

The presence of *Salmonella enterica* subspecies *enterica* serovar Typhi strains CMST (STCM), R192829 (STR1) and WG51146 (STWG) from studied samples supported the occurrence enteric *Salmonella* in the samples. Traditionally, the laboratory detection of *Salmonella* species has relied on non-selective and/or selective enrichment and subsequent culture on selective media. The introduction of molecular techniques provides a more sensitive and rapid technique for detecting these bacteria.

The highest counts of enteric *Salmonella* recorded among different ready to eat fruit salads collected from the street hawkers could be attributed to the poor handling, poor sanitation and fruits used for the preparation. Similar findings were stated by many researchers (Davies and Wales, 2018; Ali *et al.*, 2014).

5. Conclusion

The study has revealed the presence of *Salmonella enterica* subspecies *enterica* serovar Typhi strain CMCST (STCM, *Salmonella enterica* subspecies *enterica* serovar Typhi strain WG51146 (STWG) and *Salmonella enterica* subspecies *enterica* serovar Typhi strain R192829 (STR1), of which STR1 was mostly encountered in the stream samples. The present study recommends personal hygiene, community education and thorough boiling of water before use as a better means of controlling the transmission of *Salmonella* species.

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