Journal of Pollution Monitoring, Evaluation Studies and Control
J Pollut Monit Eval Stud Contr, 4(1): 95-99 (2025)
DOI: https://doi.org/10.54117/jpmesc.v4i1.17

Quotidian of Substantial Strain of *Shigella dysenteriae* among Ready-To-Eat Fruit Salad Sold in Uli Community

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Abstract

Shigella species; a dominant species found in ready-to-eat fruit salads, has been receiving drastically attention not only in causing human infections, but also for its involvement in antibiotic resistant, of which 80% of this resistant genes are encoded in the plasmid. This study was undertaken to evaluate the one-time-sampling study of *Shigella dysenteriae* among ready - to- eat fruit salads sold in Uli community. Samples were randomly collected from different vendors from different locations in Uli community using standard microbiological techniques. The prevalence of the different strains encountered in the samples were also determined. The study revealed the presence of *S. dysenteriae* strain 53-3937 (SD53), *S. dysenteriae* strain 07-3308 (SD07), *S. dysenteriae* strain BU53W (SDBU), of which 36.00% were positive for *Shigella* species and the occurrences of SD53, SD07 and SDBU were 50.00%, 19.44%, 30.56% respectively. From the above study, different strains of *Shigella* species were isolated from ready – to – eat fruit salads sold at Uli community, of which isolate SD53 was predominant strain in the study samples. The findings have shown that the ready - to - eat fruit salad samples studied were contaminated by *Shigella* species.

Keywords*: Shigella dysenteriae, Ready-to-eat fruit salads, Antibiotic resistance, Plasmid-encoded genes, Prevalence in Uli community*

Article History
Received: 02 May 2025
Accepted: 21 May 2025
Published: 27 May 2025

How to cite this paper: Iheukwumere, I. H., Iheukwumere, C. M., Obianom, A. O., Nnadozie, C. H., Okereke, F. O., Onwuasoanya, U. F., Udeagbara, O. E., Unaeze, B. C., Obiefuna, O. H., Ike, V. E., Onyemekara, N. N., & Ihenatuoha, U. A. (2025). Quotidian of Substantial Strain of Shigella dysenteriae among Ready-To-Eat Fruit Salad Sold in Uli Community. *Journal of Pollution Monitoring, Evaluation Studies and Control*, *4*(1), 95–99. https://doi.org/10.54117/jpmesc.v4i1.17

Introduction

Fruit salad is a dish consisting of various kinds of fruits, sometimes served in a liquid, either juices or syrup. In different forms, fruit salad can be served as an appetizer or a side salad. It is composed of different kinds of fresh fruits such as; apples, grapes, pineapple, watermelon, paw-paw, cucumber, coconut, etc.; which are cut into small pieces and eaten with or without milk to give it an extra flavour. Microbiological populations of fruit salad were examined and five bacteria genera were isolated which include: *Bacillus*, *Staphylococcus aureus*, *Pseudomonas*, *Escherichia* and *Streptococcus* and three fungal genera were identified to be *Penicillium spp*, *Aspergillus spp*, *Saccharomyces cerevisiae*. Ready-to-eat fruit salad is considered a food that can be eaten immediately at the point of sale without further preparation. Ready-to-eat fruit salad constitutes a suitable and appropriate meal for today's lifestyles because it needs no cooking or further preparation. Consumption of ready-to-eat fruit salads has increased worldwide (Castro-roses *et al*., 2012). Raw fruits get contaminated with pathogenic microorganisms during harvesting, post-harvesting handling, processing and distribution processes. Commonly, handling practices by street

vendors and the environment of presenting cut salads added a possibility of food-borne disease occurrences (Sadiq *et al*., 2015).

Fresh plant-origin products may be a vehicle for the transmission of bacterial pathogens including human diseases, for example; *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* (Faour-Klingbeil *et al*., 2015; Pluta *et al*., 2017), *Shigella spp*, *Staphylococcus aureus*, *Aeromonas hydrophila* and the spore-formers *Bacillus cereus*, *Clostridium botulinum* and *Clostridium perfringes* (Faour-klingbeil *et al*., 2015). Many epidemic incidence occurs due to consumption of raw vegetables contaminated by pathogenic microorganisms such as *Salmonella spp*, *Shigella spp*, *Escherichia coli* 0157 (Manhique *et al*., 2020) and *Listeria monocytogenes*. The factors that influence the growth of the microorganisms in fresh produce may include the type of organism, the commodity and environmental conditions in the field and thereafter, including storage conditions (Qadri *et al*., 2015).

The Centers for Disease Control and Prevention has described microbial contamination of ready-to-eat fruit salad as a public health concern usually in developed countries due to the presence of bacteria that are of public health concern (Abakari *et al*., 2018). *Shigella spp* has been detected regularly in surveys conducted on fresh vegetables at fresh-cut processing companies' retail establishments in different countries worldwide (Gil *et al*., 2017). *Shigella* can pollute fresh produce during production through water, soil, insects or other animals contaminated with faecal matter (de Oliveira *et al*., 2019).

Gil *et al*. (2017) specified that processing operations of fresh ready-to-eat fruit salads involve the use of many unit operations which can provide opportunities for cross-contamination. Unhygienic postharvest handling conditions could also lead to the contamination of ready-to-eat fruit salads (Ahmad *et al*., 2018).

This research work is aimed at evaluating *Shigella* species among ready-to-eat fruit salads sold in the Uli community. Ready-to-eat fruit salad can be contaminated with different types of food-borne pathogens from farm to fork that make them unsafe for human consumption. Therefore, regulatory bodies should design periodic workshop training for ready-to-eat fruit salad hawkers to help fix the problems and enhance the effectiveness of ready-to-eat fruit salad preparations.

Materials and Methods

Study Area: The study was carried out in Ihiala L.G.A, Anambra State. Ihiala is situated at Latitude 5.85ºN and Longitude 6.86ºE, with an elevation of 144 m above sea level. It is located 48 Km North of Owerri and 40 Km south of Onitsha. It covers an area of 304SqKm and is bounded by Ogbaru (in Ogbaru L.G.A, Anambra State) on the West, Ozubulu (in Ekwusigo L.G.A, Anambra State), Ukpor and Osumenyi (in Nnewi South L.G.A, Anambra State) in the North and the South by Egbuoma, Ohakpu, Ozara and Oguta in Egbema/Oguta L.G.A of Imo State. Ihiala has a tropical climate (rainy and dry seasons) with double maximal rainfall. The rainy season is between April and October, and the dry season is between November and March. The annual rainfall ranges from 1800 mm to 2000 mm. The major anthropological activities are farming/agriculture and trading, of which pig farming is one of the major farming practices. In this study, samples were collected from the major towns in Ihiala L.G.A. which included Amorka, Azia, Lilu, Okija, Mbosi, Isseke, Orsumoghu, Ubuluisiuzor and Uli.

Sample collection, handling and transportation: This was carried out using a method described in a study published by Iheukwumere *et al*. (2021). The samples used for this study were collected from the Uli community. A total of 500 ready-to-eat fruit salad samples were collected from five different villages including their markets in the Uli community. Samples were taken from five different sites, each in different containers. The ready-to-eat fruit salad samples were collected with sterile containers. The containers were thoroughly washed with detergent, rinsed with water, and then rinsed with 70% ethanol and finally rinsed three times with distilled water. The samples were carefully labelled and then kept in a disinfected cooler to maintain the temperature and stability of the number of isolates. The samples were transported to the laboratory for immediate analysis.

Isolation of organisms: This was carried out using a method described in a study published by Iheukwumere *et al*. (2021). Ten gram (10 g) ready-to-eat fruit salad sample was aseptically transferred into a sterile test tube (Pyrex) containing 100 ml of the diluent (sterile normal saline) 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 Salmonella Shigella agar medium (SSA/Biotech) using the pour plate method. All the plates were incubated and inverted at 37±2˚C for 24-48 h.

Characterization and identification of the isolates: The isolates were sub-cultured on nutrient agar (Biotech), and incubated in an inverted position at 37±2˚C for 24 hrs. The isolates were characterized and identified using their colonial and morphological descriptions as described in the study published by Iheukwumere *et al*. (2018), and biochemical reactions as described in the study published by Iheukwumere *et al*. (2020). The colonial description was carried out to determine the colours of the isolates on agar media plates, their sizes, edges, consistencies and optical properties of the isolates.

Prevalence and Distribution of the Isolates in the Ready - to - eat fruit salad Samples

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

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 a 95 % confidence level. Pair pair-wise comparison of the mean was done by the Student "t" test as described in the study published by Iheukwumere *et al* (2018) and Iheukwumere *et al*. (2020).

Results

The occurrences of the isolates in the sample are shown in Table 1. The study revealed that 39.20 % of the samples were positive for S*higella* species. Sample C showed highest occurrences of the test organism whereas sample A recorded the lowest occurrences.The cultural and morphological characteristics of the isolates is shown in Table 2. The study revealed that the Isolates exhibited different appearances on Deoxycholate citrate agar and similar elevation, Edge and surface. And also similar morphological characteristics on Gram reaction, cell morphology, endospores and motile nature. The biochemical characteristics of the isolates revealed that the isolates were Voges prokauer, Indole, Citrate, Hydrogen sulphide production, Urease, Dulcitol and Sucrose negative as shown in Table 3. The isolates differ in their variation in utilization of sugars. They were all catalase and Glucose positive but differ in their abilities to utilize Lactose, Mannitol and Inositol. The nucleic acid extracted from the isolates showed the ratio of their absorbance at wavelength of 260 nm and 280 nm. 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 isolate E, F and G were *Shigella dysenteriae* strain 53—3937 (SD53), *Shigella dysenteriae* strain 07—3308 (SD07) and *Shigella dysenteriae* strain BU53W (SDBU) as shown in Table 5. The study also revealed that SD53 showed highest occurrences in the studied sample whereas SD07 recorded the least occurrences as shown in Table 6.

Table 1: Occurrences of the Isolates in the studied samples

Sample	Number	P (%)	N (%)
A	100	27(27.00)	73(73.00)
B	100	34(34.00)	66(66.00)
C	100	43(45.00)	57(57.00)
D	100	35(35.00)	65(65.00)
E	100	57(57.00)	43(43.00)
Total	500	196(39.20)	304(60.80)

Table 2: Cultural and morphological characteristics of the isolates

Parameter	E	F	G
Appearance on DCA	Colourless/pale	Pale	Colourless
Elevation	Convex	Convex	Convex
Edge	Smooth	Smooth	Smooth
Surface	Smooth	Smooth	Smooth
Gram reaction	____	____	____
Cell morphology	Rods	Rods	Rods
Endospore	____	____	____
Motility	____	____	____

Table 3: Biochemical characteristics of the Isolates

Parameter	E	F	G
Catalase	+	+	+
Voges prokauer	_	_	_
Indole	_	_	_
Citrate	_	_	_
H_2S	_	_	_
Urease	_	_	_
Glucose	+	+	+
Lactose	+/-	_	+/-
Mannitol	+/-	+/-	+
Dulcitol	_	_	_
Sucrose	_	_	_
Inositol	_	+/-	_

Table 4: Nanodrop confirmation of nucleic acids from the Test isolates

Sample ID	Conc (Mg/Ml)	260 nm	280 nm	260/280
E	119.40	3.2271	1.7829	1.81
F	128.80	3.3522	1.8218	1.84
G	134.20	3.4220	1.8699	1.83

Table 5: Molecular identities of the isolates

Parameter	E	F	G
Max score	6076	6076	7239
Total score	6076	6076	15503
Query score (%)	100	100	100
E—value	0.0	0.0	0.0
Identity (%)	100	100	100
Accession length	4382743	4382687	184894
Accession Number	CP026780	CP026878	CP024469
Description	*Shigella dysenteriae* strain 53—3937 (SD53)	*Shigella dysenteriae* strain 07—3308 (SD07)	*Shigella dysenteriae* strain BU53W (SDBU)

Table 6: Prevalence of the isolates

Isolate	Number	Percentage (%)
SD53	18	50. 00
SD07	7	19.44
SDBU	11	30.56
Total	36	

Discussion

The consumption of ready-to-eat fruit salads directly from street vendors or hawkers potentially increases the risk of foodborne disease caused by a wide variety of pathogens due to the poor hygienic nature of the vendors and sanitary conditions of the processing environment as well as the packaging materials. Similar deductions were drawn by several researchers (Chude *et al*., 2018; Akoachere *et al*., 2018; Oblinda *et al*., 2021). Several researchers have shown that the poor hygienic nature of the vendors can introduce pathogens which contaminate the fruit salad samples (Akoachere *et al*., 2018; Uchendu *et al*., 2018; Oblinda *et al*., 2021 and Nathan-Mensah *et al*., 2024). The Sanitary conditions of the processing equipment or tools used for making fruit salad can harbour pathogens and serve as a source of contamination as reported by Mahfuza *et al*. (2016).

The presence *Shigella dysenteriae* strain 53-3937 (SD53), *Shigella dysenteriae* strain 07-3308 (SD07), *Shigella dysenteriae* strain BU53W (SDBU) in the studied ready-to-eat fruit salad samples supported the findings of many researchers (Tehrani *et al*., 2018; Olaimat *et al*., 2020; Berihu *et al*., 2024). Tehrani *et al*. (2018) stated in their report that *Shigella* spp. was mostly found as the principal contaminant of fruit salad and also responsible for foodborne disease due to the production of Shiga toxins.

The vendors, water and inadequate washing of hands and utensils appear to be the major hazard associated with these fruits and must be addressed properly (Oblinda *et al*., 2021). Vendors and consumers are advised to wash fruits properly before peeling, slicing or cutting; fruits should be handled with clean and sanitized hands, utensils and surfaces and also stored refrigerated if any delay before consumption. Good personal hygiene and effective hazard analysis and critical control point (HACCP) application reduce the chance of contamination of ready–to–eat fruit salads.

Conclusion

The study revealed the presence of *Shigella dysenteriae* strain 53-3937 (SD53), *Shigella dysenteriae* strain 07 – 3308 (SD07), *Shigella dysenteriae* strain BU53W (SDBU), of which SD53 was mostly encountered in the ready – to – eat fruit salad samples. The present study recommends personal hygiene, community education and thorough washing of fruits before use as a better means of controlling the transmission of *Shigella* species.

Acknowledgment: We are grateful to all our study participants who join the study voluntarily. We are grateful to ZAHARM Analytical and Research Laboratory, Amawbia, Awka Anambra State, Nigeria for providing enabling environment, resources and techniques for this study. We really salute their wonderful efforts.

Conflict of interests: The authors declare that they have no conflict of interests.

Funding: This research did not receive specific grant from any funding agencies.

Ethical approval: Not applicable

Authors Contributions: All contributed towards the study design, experiment execution, data analysis, and manuscript drafting.

Availability of Data and Materials: All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

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