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Research Article

Detection of OrgA Gene in *Salmonella* Species Isolated from Selected Ready-To-Eat (RTE) Sliced Fruits Sold in Kano Municipal LGA, Kano - Nigeria

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ABSTRACT

This study investigated the presence of the OrgA gene in *Salmonella* species isolated from ready-to-eat (RTE) sliced fruits sold in Kano Municipal LGA, Nigeria. The objectives were to determine the total mesophilic aerobic and *Salmonella* counts, isolate and characterize *Salmonella* spp., detect the OrgA gene in the isolates, and assess the pathogenicity of OrgA-positive strains. A total of 40 RTE sliced fruit samples (09 pawpaw, 12 pineapple, and 19 watermelon) were analyzed using microbiological, biochemical, and molecular techniques. The results showed that pawpaw samples had the highest bacterial count (8.10×10^4 to 9.45×10^5 CFU/g) and *Salmonella* count (5.50×10^1 to 1.40×10^2 CFU/g), followed by watermelon (bacterial count: 1.90×10^5 to 3.56×10^5 CFU/g; *Salmonella* count: 0 to 1.14×10^2 CFU/g) and pineapple (bacterial count: 1.02×10^5 to 9.05×10^5 CFU/g; *Salmonella* count: 0 to 7.10×10^1 CFU/g). Molecular analysis confirmed the presence of the OrgA gene in *Salmonella* isolates from pawpaw and pineapple, indicating the occurrence of virulent strains. Pathogenicity assessment showed that OrgA-positive *Salmonella* isolates survived and multiplied in fruits, causing spoilage and fulfilling Koch's postulates. These findings highlight the public health risks associated with consuming contaminated RTE sliced fruits, particularly in environments with poor hygiene. The study emphasizes the need for improved food safety measures, regular microbial surveillance, and enforcement of strict hygiene regulations to minimize *Salmonella* contamination in fresh produce.

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INTRODUCTION

The rise in foodborne illnesses has been a growing concern, particularly in developing countries where food safety regulations may be less stringent, and public awareness about food hygiene practices is often limited. In such contexts, the prevalence of *Salmonella* spp. in various food items, including ready-to-eat (RTE) foods, has become a significant public health issue (Harrison *et al.*, 2022). RTE foods are particularly susceptible to contamination because they do not require further cooking before consumption, which would otherwise kill any harmful pathogens present. Among the various RTE foods, sliced fruits are especially vulnerable due to their high water content and the extensive handling they undergo during preparation and sale (Hölzel *et al.*, 2018).

Recent studies have shown that *Salmonella* spp. isolated from food sources in Nigeria are increasingly exhibiting multidrug resistance, making infections more difficult to treat. The presence of virulence genes, such as the OrgA gene, in *Salmonella* strains further complicates the situation (Ulger *et al.*, 2018). The OrgA gene is a component of the *Salmonella* Pathogenicity Island 1 (SPI-1), which encodes a type III secretion system (T3SS) essential for the bacterium's ability to invade host cells and cause disease. The detection of the OrgA gene in *Salmonella* isolates from RTE foods would indicate the presence of potentially highly virulent strains capable of causing severe infections in consumers (Pérez-Rodríguez and Mercanoglu, 2019).

The results of this study will have significant implications for public health and food safety in Kano metropolis and beyond. By identifying the presence of virulent *Salmonella* strains in RTE sliced fruits, this research will provide evidence-based data that can inform food safety policies and interventions aimed at reducing the risk of foodborne illnesses. Furthermore, the detection of the OrgA gene in *Salmonella* isolates will contribute to the understanding of the molecular mechanisms underlying the pathogenicity of these bacteria, which is essential for the development of new strategies to combat *Salmonella* infections. Therefore, this research aims to address a critical public health issue by investigating the presence of *Salmonella* spp. and the OrgA gene in RTE sliced fruits sold in Kano metropolis. The outcomes of this study will provide valuable insights into the

microbiological quality of these products and contribute to the ongoing efforts to improve food safety in Nigeria.

2.0 MATERIALS AND METHODS

A total of 40 ready-to-eat (RTE) sliced fruit samples comprising pawpaw, pineapple, and watermelon were randomly collected from various vending outlets across Kano Municipal LGA, Nigeria. The samples were aseptically packaged in sterile containers and transported in iceboxes to the laboratory within six hours for microbiological analysis. For each sample, 25 grams were homogenized in 225 mL of buffered peptone water and pre-enriched by incubation at 37°C for 18 ± 2 hours (Park *et al.*, 2021). Serial dilutions up to 10⁻⁵ were prepared, and aliquots were plated on nutrient agar and *Salmonella*-*Shigella* agar to determine total viable and *Salmonella* counts, respectively. Following incubation at 37°C for 24 hours, colonies within the range of 30–300 CFU/mL were counted. Suspected *Salmonella* colonies were further enriched in Rappaport Vassiliadis broth and plated on selective media, with presumptive isolates characterized by morphological, Gram staining, and a battery of biochemical tests including motility, citrate utilization, urease, methyl red-Voges Proskauer, H₂S production, lysine iron agar, and indole tests (Parker *et al.*, 2021; Manageiro *et al.*, 2020).

Pathogenicity of the isolates was assessed through Koch's postulates by inoculating sterilized fruit samples with *Salmonella* suspensions (~10⁸ CFU/mL) and observing for spoilage over 1–7 days under ambient conditions. Molecular identification involved genomic DNA extraction using a modified lysis protocol (Richter *et al.*, 2019), followed by PCR amplification of *invA* and *orgA* genes using specific primers. Amplification of the *orgA* gene—a critical virulence determinant located in *Salmonella* Pathogenicity Island-1 (SPI-1)—was performed with an annealing temperature of 55°C, targeting a 255 bp product (Klingl *et al.*, 2020). PCR products were analyzed via agarose gel electrophoresis using safe dye visualization under UV light. Statistical analysis of microbial counts and gene detection outcomes was conducted using SPSS v21, applying ANOVA and Duncan's multiple range test at a 5% significance level (Parisi *et al.*, 2020).

3.0 RESULTS AND DISCUSSIONS

The study revealed significant microbial contamination in the analyzed ready-to-eat (RTE)

sliced fruits sold in Kano Municipal LGA. Among the 40 fruit samples assessed, pawpaw had the highest total bacterial load, ranging from 8.10×10^4 to 9.45×10^5 CFU/g, and *Salmonella* counts between 5.50×10^1 and 1.40×10^2 CFU/g. Watermelon and pineapple followed, with varying levels of bacterial and *Salmonella* counts. These findings are consistent with previous reports indicating that RTE fruits, especially those with high moisture content and exposed surfaces, provide a favorable environment for microbial proliferation when hygiene standards are poor (Gosling *et al.*, 2018; Liu *et al.*, 2022). The observed contamination levels highlight the health risks posed by consuming improperly handled or stored fresh-cut fruits in public markets and street-vending settings.

Biochemical and cultural identification confirmed the presence of *Salmonella* spp. in several samples,

which were further subjected to molecular screening. PCR analysis detected the *orgA* gene in isolates from both pawpaw and pineapple samples, indicating the presence of virulent *Salmonella* strains. The *orgA* gene is associated with the *Salmonella* Pathogenicity Island 1 (SPI-1), which encodes the type III secretion system (T3SS) responsible for bacterial invasion of host cells (Wei *et al.*, 2020; Rosario *et al.*, 2022). The detection of this gene suggests that some of the *Salmonella* strains found in the RTE fruits have a high pathogenic potential, capable of causing severe gastroenteritis and possibly systemic infections, particularly in vulnerable individuals. These results align with studies conducted in other parts of Nigeria and sub-Saharan Africa that reported similar occurrences of virulence genes in foodborne *Salmonella* isolates (Mesbah *et al.*, 2019).

Table 1. Mean Bacterial and *Salmonella* Counts from the Sampled Ready-To-Eat (RTE) Sliced Fruits Sold in Kano Municipal LGA, Kano – Nigeria based on Sample's Location

Fruits	Location	BC \pm SD (CFU/g)	SC \pm SD (CFU/g)
Pawpaw	Zango	$4.55 \times 10^5 \pm 1.33 \times 10^{5a}$	$1.70 \times 10^2 \pm 3.00 \times 10^{1a}$
	Sharada	$8.10 \times 10^5 \pm 3.90 \times 10^{4a}$	$1.00 \times 10^2 \pm 1.00 \times 10^{1b}$
	Gandun Albasa	$3.41 \times 10^5 \pm 2.69 \times 10^{5a}$	$1.25 \times 10^2 \pm 5.00^a$
	Dan'agundi	$3.62 \times 10^5 \pm 1.26 \times 10^{5a}$	$1.80 \times 10^2 \pm 3.16 \times 10^{1c}$
	Yakasai	$4.83 \times 10^5 \pm 1.79 \times 10^{5a}$	$2.35 \times 10^2 \pm 2.59 \times 10^{1a}$
	Shahuuci	$2.31 \times 10^5 \pm 2.00 \times 10^{4a}$	$1.35 \times 10^2 \pm 1.50 \times 10^{1a}$
Pine Apple	Zango	$2.72 \times 10^5 \pm 3.60 \times 10^{4a}$	$7.10 \times 10^2 \pm 7.78^a$
	Sharada	$2.54 \times 10^5 \pm 4.25 \times 10^{4a}$	$2.50 \times 10^2 \pm 1.77 \times 10^{1b}$
	Gandun Albasa	$1.92 \times 10^5 \pm 9.00 \times 10^{3a}$	$7.65 \times 10^2 \pm 9.55 \times 10^{1a}$
	Dan'agundi	$2.45 \times 10^5 \pm 4.50 \times 10^{3a}$	$1.90 \times 10^2 \pm 1.34 \times 10^{1b}$
	Yakasai	$2.28 \times 10^5 \pm 6.50 \times 10^{3a}$	$3.90 \times 10^2 \pm 2.76 \times 10^{1a}$
	Shahuuci	$2.70 \times 10^5 \pm 3.65 \times 10^{4a}$	$7.15 \times 10^2 \pm 1.06 \times 10^{1a}$
Water Melon	Zango	$3.32 \times 10^5 \pm 1.35 \times 10^{4a}$	$7.83 \times 10^2 \pm 1.53 \times 10^{1a}$
	Sharada	$3.04 \times 10^5 \pm 5.16 \times 10^{4a}$	$1.05 \times 10^2 \pm 9.50^a$
	Gandun Albasa	$3.11 \times 10^5 \pm 3.37 \times 10^{4a}$	$5.72 \times 10^2 \pm 1.11 \times 10^{1a}$
	Dan'agundi	$3.10 \times 10^5 \pm 3.14 \times 10^{4a}$	$2.32 \times 10^2 \pm 4.01 \times 10^{1b}$
	Yakasai	$3.11 \times 10^5 \pm 5.75 \times 10^{4a}$	$2.83 \times 10^2 \pm 4.91 \times 10^{1a}$
	Shahuuci	$2.77 \times 10^5 \pm 8.74 \times 10^{4a}$	$6.26 \times 10^2 \pm 4.45 \times 10^{1a}$

Key Words; Mean \pm SD with different superscripts differ significantly at the p(0.05) level.

Table 2. Prevalence of *invA* and *orgA* genes based on the sample's locations and fruits types

Location	Pawpaw	Pine Apple	Water melon
Zango	<i>orgA</i>	-	-
Sharada	-	<i>orgA</i>	-
Gandun Albasa	<i>orgA</i>	-	-
Dan'agundi	-	-	-
Yakasai	<i>invA</i>	-	-
Shahuuci	-	<i>invA</i>	-

Keys; (+) – *OrgA* present, (-) – *OrgA* absent

Pathogenicity testing further validated the virulence of the *orgA*-positive strains. Inoculated fruits developed spoilage symptoms including discoloration and softening, and re-isolation of *Salmonella* from these samples fulfilled Koch's postulates. This confirmed the ability of the *Salmonella* strains not only to survive and multiply in fruit tissue but also to initiate infection-like conditions. These outcomes reinforce the urgency for improved food safety interventions, especially in informal food sectors. As emphasized by Karanth *et al.* (2022) and Possas *et al.* (2023), the implementation of microbial monitoring, vendor education, and enforcement of hygiene practices is essential to reducing the incidence of foodborne illnesses. The study also recommends future research on antimicrobial resistance profiling and surveillance to better understand and mitigate the risks posed by virulent and potentially drug-resistant *Salmonella* strains in fresh produce.

Conclusion

In conclusion, this study confirms the presence of *Salmonella* species harboring the *orgA* virulence gene in ready-to-eat (RTE) sliced fruits sold in Kano Municipal LGA, highlighting a significant public health risk associated with their consumption. The detection of the *orgA* gene, which plays a key role in the invasion and pathogenicity of *Salmonella*, indicates that some isolates possess enhanced virulence potential capable of causing severe foodborne illnesses. The findings underscore the urgent need for improved hygiene practices during the processing, handling, and vending of RTE fruits, as well as routine microbial surveillance and public health education to reduce contamination and disease transmission. Strengthening regulatory enforcement and investing in food safety infrastructure are essential steps toward safeguarding consumers and preventing future outbreaks linked to contaminated fresh produce.

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