Detection of *Serratia marcescens* in Sliced Fruit Sold in Yakuba, Ilorin, Kwara State, Nigeria

Adedayo R. Majekodunmi[1], and Mohammed Jibril[2*]

[1]Department of Microbiology, Faculty of Pure and Applied Sciences, Kwara State University, Malete, Kwara State, Nigeria.
[2]Clinical Services Department, Diagnostic Unit, Federal Neuro-Psychiatric Hospital, Budo-Egba.

CORRESPONDENCE
Jibril Mohammed
Clinical Services Department,
Diagnostic Unit, Federal Neuro-
Psychiatric Hospital, Budo-Egba
jibrilmohammed2001@gmail.com
+234-816-996-1623

CONFLICT OF INTEREST: None
ETHICAL APPROVAL: Not Applicable

This is a publication of the
Nigerian Stored Products
Research Institute (NSPRI)

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Abstract

Sliced fruits are widely consumed for their nutritional benefits; however, improper handling can lead to microbial contamination, posing public health risks. Serratia marcescens is an opportunistic pathogen capable of transmission through contaminated food. This research focused on detecting the presence of Serratia marcescens in sliced fruits sold in the Yakuba area of Ilorin. A total of 50 samples comprising watermelon (36%), pawpaw (34%), and pineapple (30%) were collected from six vendors. Isolation and identification of Serratia marcescens were performed using standard microbiological techniques. Antibiotic susceptibility testing was also conducted using the Kirby-Bauer disc diffusion method. Two isolates were identified as Serratia marcescens, accounting for 4% of all samples; both were found in watermelon, giving a fruit-specific prevalence of 11.11% (2/18). No contamination was detected in pawpaw or pineapple samples. The contaminated samples originated exclusively from Vendor 3, resulting in a vendor-specific contamination rate of 22.22%. Antibiotic susceptibility testing showed that the isolates were 100% susceptible to ceftriaxone, trimethoprim-sulfamethoxazole, gentamicin, and tetracycline but showed complete resistance to ampicillin. In conclusion, the sliced fruit samples were contaminated with Serratia marcescens, and the isolates were resistant to one of the commonly used antibiotics.

Keywords:

Introduction

Fruits are an essential source of vital nutrients such as vitamins, minerals, antioxidants, and phytonutrients. As crucial components of a balanced diet, they are necessary in health promotion and preventing numerous diseases (Abdulkareem & Odeh, 2021). Sliced fruits served by Nigerian street vendors are usually chosen for their affordability, accessibility, and convenience. However, such ready-to-eat (RTE) foods often lack adequate food safety measures, rendering them particularly vulnerable to microbial contamination through slicing and handling procedures (Odebisi-Omokanye et al., 2015). RTE fruits are typically consumed without further processing, such as washing or cooking, which increases the risk of food-borne diseases. The World Health Organization estimates that nearly 600 million food-borne illness cases happen worldwide yearly, resulting in an estimated 420,000 deaths, with the highest incidence being in children under the age of five years (WHO, 2015). Fruit contamination can take place at various levels, starting from the point of harvest to post-harvest handling, and is usually worsened by poor hygiene, use of contaminated water, poor storage, and contact with environmental contaminants (Micheal et al., 2022; Oladosu et al., 2023; Omorodion & Nwiyege, 2023). *Enterobacteriaceae* family members are regularly asso-

ciated with instances of contamination because they are ubiquitous in the environment and can easily colonise food surfaces during handling and storage (Ajiboye & Emmanuel, 2021). Sliced fruits contaminated with bacteria like *Salmonella* spp., *Shigella* spp., and *Escherichia coli* have been reported (Ajao et al., 2022).

Among numerous food-borne pathogens, *Serratia marcescens* is an emerging and comparatively less well-documented organism of particular concern (Abbas & Hagazy, 2020). It is generally considered an indicator of unhygienic and unsanitary habits (Khayat et al., 2023). A Gram-negative, facultative anaerobic member of the *Enterobacteriaceae* family, *Serratia marcescens,* was previously considered a non-pathogenic environmental microbe. It is, however, now known as an opportunistic pathogen associated with nosocomial infections like urinary tract infection, pneumonia, sepsis, and wound infections, particularly in immunocompromised individuals (Radeva et al., 2023; Chen et al., 2025). Its significance in public health has grown due to its intrinsic and acquired resistance to various antibiotics, including β-lactams and aminoglycosides (Roy et al., 2023). In addition, *Serratia marcescens* can create biofilms on abiotic surfaces, including knives, cutting boards, and water containers (Rice et al., 2005). This contributes to its survival in food environments and reduces its vulnerability to disinfection (Carrascosa et al., 2021). These attributes render it a potentially hazardous contaminant in informal food environments where hygiene and sanitation are inadequately implemented. While it is primarily linked with nosocomial infections, *Serratia marcescens* has been isolated from various food types, such as dairy products, vegetables, and fruits (Akhtar et al., 2016; Korcz et al., 2021; Claussen and Schmid, 2023). Despite its significance, limited information regarding its occurrence in ready-to-eat sliced fruits in Nigeria is available.

The Yakuba area of Ilorin, Kwara State, is a densely populated region, especially among the student populace, where street vending fruits and other food items is common. Given the potential health threat presented by contaminated fruits and the limited monitoring of newly emerging pathogens such as *Serratia marcescens*, there is a need to assess the microbiological safety of the commonly consumed food items. Therefore, the study aimed to detect and characterise *Serratia marcescens* in sliced fruits being sold in Yakuba, Ilorin. The findings of this study will give data on the prevalence of this organism in street-vended fruits and contribute to strategies to improve food safety and public health in the region.

Materials and Methods
Study area
This study was conducted in Yakuba, a densely populated area in Ilorin, Kwara State, Nigeria. Geographically, Yakuba is located at approximately latitude 8.55630º N and longitude 4.65000º E. The location is specifically known for having a high population of students and street vending, especially with ready-to-eat (RTE) fruits. The research work was carried out to assess the microbiological quality of sliced fruits sold by vendors along the major streets and junctions in the area.

Sample collection
Fifty sliced fruit samples were collected from six different vendors in the Yakuba area. The sample size was determined based on sampling feasibility and area coverage. The fruits included commonly vended watermelon, pawpaw, and pineapple. Each sample was placed into a sterile, clearly labelled container and immediately stored on ice. This was done to minimise bacterial growth while transporting the samples to the Biofare Microbiology Laboratory for analysis. The samples were coded as F1–F50 respectively.

Cleaning and disinfection of glassware and laboratory bench
All glasswares used during the experiment were washed with detergent and potable tap water, then rinsed with distilled water. They were sterilised using a hot air oven at 170 °C for 1 hr. The laboratory bench was wiped with 70% ethanol before and after every experimental step to prevent cross-contamination and maintain aseptic conditions.

Isolation of bacteria
Ten gram of each fruit samples were aseptically weighed and homogenised in 90 mL sterile distilled water to a 1:10 dilution which was further serially diluted to 10^{-3}. Aliquots (0.1 mL) were taken from the 10^{-2} dilution and spread onto modified Oxford agar plates supplemented with 0.5% yeast extract and 0.1% caprylic acid, and incubated at 37 °C for 24 hours. Following incubation, distinct red-pigmented colonies characteristic of *Serratia marcescens* were subcultured onto fresh agar plates to obtain pure isolates for further analysis.

Morphological characterisation and gram staining of *Serratia marcescens* isolates

Initially, colonies suspected to be *Serratia marcescens* were analysed through distinguishable morphological features, such as shape, size, colour, surface texture, height, and edge definition. Then Gram staining was performed to observe the Gram reaction and cell shape. A thin smear of each bacterial isolate was made on a sterile glass slide, heat-fixed, stained successively with crystal violet for 1 minute, washed with distilled water. The smear was then exposed to iodine solution for 1 minute, washed and decolourised briefly with acetone-alcohol for approximately 10 seconds. Then safranin was added for 30 seconds before final rinsing. The resulting stained smear was observed using a light microscope (Olympus CX-23) with an oil immersion objective (Willey et al., 2014).

Biochemical identification of *Serratia marcescens* isolates

The isolates were subjected to biochemical tests to confirm *Serratia marcescens.* Following established protocols, the tests comprised catalase, oxidase, citrate, indole, Voges-Proskauer, and urease tests. For the catalase test, a drop of 3% hydrogen peroxide was added to a portion of the bacterial colony on a clean slide to observe for effervescence (Reiner, 2010). The oxidase test was conducted by smearing the bacterial colony on filter paper pre-soaked with oxidase reagent to detect any colour change (Shields & Cathcart, 2010). Indole production was tested using peptone water supplemented with tryptophan and the addition of Kovac's reagent after incubation (MacWilliams, 2012). Methyl red and Voges-Proskauer tests were performed using glucose phosphate broth, followed by adding methyl red indicator and Barritt's reagents (A and B), respectively (McDevitt, 2009). Citrate utilisation was assessed by inoculating the isolates on Simmons citrate agar slants (Hamza & Alebejo, 2017), while urease activity was evaluated using urea agar slants (Brink, 2010). The isolates were confirmed using standard biochemical characteristics and Bergey's Manual of Determinative Bacteriology.

Antibiotic susceptibility testing

Antibiotic susceptibility profile of the *Serratia marcescens* isolates was evaluated using the Kirby-Bauer disk diffusion method. Fresh isolates were suspended in sterile 0.85% normal saline using a sterile cotton swab and adjusted to match the turbidity of a 0.5 McFarland standard (1.5×10^8 CFU/mL) by visually comparing the commercial 0.5 McFarland standard (Hardy Diagnostics, USA) and the organism suspension using a Wickerham card (white background with a black line) to standardise it. The suspension is adjusted by adding more saline or culture until the turbidity matches that of the 0.5 McFarland standard. The standardised inoculum was spread evenly over the surface of sterile Mueller-Hinton agar (HIMEDIA M173-500G) plates using a sterile swab. The agar was prepared according to the manufacturer's specification (38 g in 1000 mL) and sterilised at 121 °C for 15 minutes. Commercially prepared antibiotic discs were aseptically placed onto the inoculated plates using sterile forceps. The plates were incubated at 37 °C for 18 hours. Following incubation, the diameters around each disc were measured in millimetres using a ruler. Results were recorded as sensitive, intermediate, or resistant according to CLSI guidelines. The antibiotics tested were ceftriaxone, trimethoprim-sulfamethoxazole, gentamicin, tetracycline, and ampicillin (CLSI, 2020).

Data analysis

Data analysis was carried out using SPSS software, version 25.0. Descriptive statistics were applied to determine the prevalence of *Serratia marcescens* among sliced fruit samples. The Chi-square test assessed potential associations between contamination rates and variables such as fruit type and vendor. A p-value below 0.05 was interpreted as statistically significant.

Results and Discussion
Sample distribution
The distribution of samples by fruit type and vendor is shown in Table 1. The most represented was watermelon, with 36% (n = 18) of the sample population, closely followed by pawpaw at 34% (n = 17), and the least represented was pineapple at 30% (n = 15). All vendors provided at least two samples per distinct fruit type, with Vendors 1-3 providing three samples per fruit type, and Vendor 6 providing a slightly smaller total number of samples (n = 7). This range reflects the general patterns of availability and vendor participation over the sampling period for this study.

Characterisation and identification of *Serratia marcescens* isolates.

Two isolates with red colour on agar media were observed among the 50 fruit samples examined and were tentatively identified as *Serratia marcescens*. Both isolates had identical colony characteristics:

circular, smooth, red-pigmented colonies with whole margins and convex shape. Microscopy confirmed the isolates to be Gram-negative bacilli. Biochemical tests showed that the isolates were catalase, Voges-Proskauer, citrate, and urease positive, but oxidase, indole, and methyl red tests were negative.

Collectively, these features are consistent with the established profile of *Serratia marcescens*. As the two isolates shared similar properties, Table 2 shows a representative profile. The isolation of *Serratia marcescens* in this study is supported by the work of Akhtar et al. (2016).

Table 1: Distribution of Samples by Fruit Type and Vendor

Vendor ID	Sample			
	Watermelon	Pawpaw	Pineapple	Total Samples
V1	3	3	3	9
V2	3	3	3	9
V3	3	3	3	9
V4	3	3	2	8
V5	3	3	2	8
V6	3	2	2	7
Total	18	17	15	50
Percentage of Fruit Type (%)	36	34	30	100

Table 2: Morphological Characteristics, Gram's Reaction, and Biochemical Characteristics of *Serratia marcescens* Isolates

Characteristics	Observations
Morphology	
Shape	Circular
Size	Medium
Color	Red
Texture	Smooth
Elevation	Convex
Margin	Entire
Gram's Reaction	Negative
Biochemical	
Catalase	Positive
Oxidase	Negative
Indole	Negative
Methyl Red	Negative
Voges Proskauer	Positive
Citrate	Positive
Urease	Positive

Prevalence of *Serratia marcescens* by fruit type

The prevalence of *Serratia marcescens* among the fruits analysed is given in Table 3. Among all fruit types, contamination was only observed in watermelon samples, with a prevalence rate of 11.11% (2 of 18). No contamination of *Serratia marcescens* was observed in either pawpaw or pineapple samples, leading to an overall contamination rate of 4% in all the samples analysed. Statistical analysis using the chi-square test revealed no significant association between fruit type and *Serratia marcescens* (p = 0.157). Although only watermelon was contaminated, the difference was not statistically significant. The low number of positive samples may reflect relatively good hygiene among most vendors or fruits' innate resistance to external microbial contamination under certain conditions.

Nevertheless, any level of contamination is of concern, especially for bacteria that carry different virulence factors and cause opportunistic infections. *Serratia marcescens* is recognised for its expression of various virulence factors that augment its pathogenic potential. Among these factors are the synthesis of extracellular enzymes, including proteases (serralysin), hemolysins, and lipases, which aid in tissue invasion and the evasion of host immune responses (Khalil et al., 2024). Additionally, this bacterium can develop biofilms on surfaces, promoting its survival in environmental reservoirs and its capacity to resist sanitising interventions (Rice et al., 2005). These features make

Serratia marcescens a significant concern in clinical and food-related environments, with a risk of cross-contamination. Notably, watermelon, the only fruit type contaminated with *Serratia marcescens* in this current research, indicates that watermelon, among the fruits examined, may be more prone to contamination. However, the statistical analysis did not support this hypothesis. The physical nature of the fruit, particularly its high water content, may offer conditions for bacterial growth. The lack of significant correlation may be due to various factors, including the small number of positive samples (only two positive watermelon samples). The results from the statistical analysis indicate that the contamination with *Serratia marcescens* is not strictly dependent on the type of fruit, i.e., the risk could be equal across various fruits.

Prevalence of *Serratia marcescens* by Vendor
Serratia marcescens was isolated only from samples obtained from Vendor 3, representing a vendor-specific prevalence of 22.22% (2/9) (Table 4). No isolates were detected in samples taken from the other five vendors. The distribution suggests the contamination may be linked to specific handling or environmental conditions unique to Vendor 3. It is possible that the vendor introduced the contamination through poor handling practices, including using contaminated knives or cutting boards during fruit preparation, or possibly from the fruit coming into contact with contaminated surfaces such as soil or dirty tables during display. Cross-contamination from hands, utensils, or surfaces could easily transfer *Serratia marcescens* to the fruits, particularly due to the soft and moist texture of watermelon pulp, which can easily support the growth of bacteria. Issues of poor handling of food and poor hygiene among vendors have been identified as a major cause of microbial contamination in ready-to-eat fruits, as reported by Ajiboye & Emmanuel (2021). Despite this observed association, the Chi-Square test did not reveal a statistically significant relationship between vendor and contamination, with a *p*-value of 0.091. This indicates that contamination does not appear vendor-specific and that all vendors are equally likely to be contaminated, even though only Vendor 3 had positive samples. The absence of statistically significant correlation between the vendor and contamination can also be attributed to the fact that there were remarkably few positive samples, and this restrains the test from identifying an actual difference.

Antibiotic susceptibility profile of *Serratia marcescens* isolates
The antibiotic susceptibility profiles of the two *Serratia marcescens* are presented in Table 5. Both isolates were susceptible to ceftriaxone, trimethoprim-sulfamethoxazole, gentamicin, and tetracycline, with inhibition zones between 23 mm and 30 mm. Conversely, both isolates demonstrated resistance to ampicillin, as indicated by the reduced inhibition zones measuring 12 mm and 13 mm. The uniform resistance observed to ampicillin may suggest a shared resistance mechanism among the isolates, highlighting the need for keeping resistance patterns among local bacterial populations under surveillance, especially in food-borne pathogens. The resistance to beta-lactam antibiotics, as indicated by ampicillin, is frequently mediated through the production of beta-lactamases, an activity that has been extensively reported in *Serratia marcescens* (Abbas & Hegazy, 2020; Claussen & Schmid, 2023). The susceptibility to other antibiotics, such as ceftriaxone and gentamicin, indicates that *S. marcescens* in this study can still be handled with other commonly used antibiotics. The antibiotic susceptibility pattern in this study is consistent with previous studies that have reported various susceptibility patterns of *Serratia marcescens* to different antibiotics. For instance, the susceptibility of *Serratia marcescens* isolates to gentamicin and resistance to ampicillin is supported by the work of Awoderu & Omololu-Aso (2021).

Table 3: Prevalence of *Serratia marcescens* by Fruit Type

Fruit Type	Total Samples	Positive Samples	Prevalence (%)
Watermelon	18	2	11.11
Pawpaw	17	0	0.0
Pineapple	15	0	0.0
Total	50	2	4.0

Chi-Square: The results showed no significant association (($\chi^2(2) = 3.704$, $p = 0.157$))

Table 4: Prevalence of *Serratia marcescens* by Vendor

Vendor ID	Total Samples	Positive Samples	Prevalence (%)
Vendor 1	9	0	0.0
Vendor 2	9	0	0.0
Vendor 3	9	2	22.22
Vendor 4	8	0	0.0
Vendor 5	8	0	0.0
Vendor 6	7	0	0.0
Total	**50**	**2**	**4.0**

Chi-Square: The results showed no significant association (($\chi^2 (5) = 9.491$, $p = 0.091$))

Table 5: Antibiotic Susceptibility Profile of *Serratia marcescens* Isolates

Antibiotics	Disc content (µg)	Isolate 1		Isolate 2	
		Zone of inhibition (mm)	Interpretation	Zone of inhibition (mm)	Interpretation
Ceftriaxone	30	30	Susceptible	29	Susceptible
Trimethoprim-Sulfamethoxazole	25	28	Susceptible	27	Susceptible
Gentamicin	10	26	Susceptible	24	Susceptible
Tetracycline	30	23	Susceptible	24	Susceptible
Ampicillin	10	13	Resistant	12	Resistant

Conclusion

The detection of *Serratia marcescens* in ready-to-eat sliced watermelon on sale in the Yakuba area of Ilorin underscores the potential risk of microbial contamination of ready-to-eat sliced fruit. Although the overall prevalence was low, the isolation of *Serratia marcescens* still emphasises the need for stringent hygiene practices in selling and handling fruits. The result obtained in this study showed that the contamination is fruit and vendor-specific; however, the lack of statistically significant association between contamination and either fruit type or vendor indicates that the contamination risk is neither fruit-specific nor supplier-specific, and that all ready-to-eat sliced fruits are vulnerable. Also, the antibiotic susceptibility profile demonstrating resistance to ampicillin underscores the necessity for continuous monitoring to prevent the spread of antibiotic-resistant strains through the food chain. These findings collectively emphasise the pressing need for improved food safety awareness and practices at all fruit-handling levels, from vendors to consumers. It would be suggested based on the research that the vendors be trained on hygiene and food handling practices, sliced fruits be stored at proper temperatures to prevent bacterial growth, regular checks on vendors be conducted to ensure compliance with food safety and consumers be informed about the risks and washing of the sliced fruits.

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