Original Research Article

Nigerian Journal of Biotechnology and Life Sciences
ISSN: 2814-0281
Vol. 3 No 2 (2026) p. 44 - 51
http://www.njbls.fud.edu.ng/

Assessment of the Microbiological Quality and Antibiotic Susceptibility Profile of Bacterial Pathogens Associated with Spoilt Oranges (*Citrus sinensis*) and Watermelons (*Citrullus lanatus*) Sold in Dutse, Northern- Nigeria

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https://doi.org/10.67601/njbls.v3i2.23

Article into
Date received
12/06/2026
Date accepted
14/07/2026
Available online
04/09/2026

Keywords
Microbial contamination, *Citrus sinenesis*, *Citrus lanatus*, Antibiotic resistance, Bacterial isolates, Food safety

Abstract

Fresh fruits such as oranges and watermelons are vital components of the human diet due to their rich nutritional content. However, these fruits can serve as vehicles for pathogenic microorganisms, especially when exposed to unhygienic handling during harvesting and storage. This study was carried out to assess the microbial quality of oranges and watermelons where approximately 10g of spoilt fruit tissue was aseptically excised from each sample using a sterile scalpel. Twenty fruit samples (10 oranges and 10 watermelons) were randomly collected from local vendors. Standard microbiological techniques were employed to enumerate bacterial loads using serial dilution and pour plate methods. Bacterial isolates were identified using Gram staining and biochemical tests, while antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method. The total viable bacterial counts ranged from 1.3×10^4 to 2.15×10^5 CFU/mL. The highest bacterial load was found on orange sample S4 (2.15×10^5 CFU/mL). Both Gram-positive and Gram-negative bacteria were isolated, with *Staphylococcus spp.*, *Streptococcus spp.*, *Bacillus spp.*, *Escherichia coli*, *Pseudomonas spp.*, and *Klebsiella spp.* being the predominant organisms. *Pseudomonas spp.* had the highest occurrence in watermelon samples (33%), while *Staphylococcus spp.* and *Pseudomonas spp.* were most prevalent in orange samples (30% each). Antibiotic susceptibility testing revealed multidrug resistance, with *E. coli* and *Klebsiella spp.* being resistant to nalidixic acid and sulfamethoxazole, while *Staphylococcus spp.* was sensitive to vancomycin and novobiocin. These results highlight the need for improved hygiene

in fruit handling and point to potential public health risks from consuming contaminated fresh produce.

Introduction

Fruits are an indispensable component of the human diet, serving as a rich source of vitamins, minerals, antioxidants, and dietary fiber (Barth et al., 2013). They play a central role in promoting health and reducing the risk of chronic diseases such as cardiovascular disorders, gastrointestinal cancers, and micronutrient deficiencies. Among these, oranges (*Citrus sinensis*) and watermelons (*Citrullus lanatus*) are particularly important in Nigeria due to their high consumption, economic value, and year-round availability (Tafinta et al., 2013). Oranges are valued for their vitamin C content, flavonoids, and contribution to immune health, while watermelons are renowned for their high-water content, lycopene, and refreshing properties in hot climates (FAO, 2020; Adeyemi et al., 2021). Post-harvest contamination of fruits is a global problem, but it is particularly severe in developing countries where poor handling, inadequate storage, high ambient temperatures, and weak food safety enforcement contribute to high levels of microbial spoilage (Chukwuka et al., 2010; Oyeleke & Akinlabi, 2019).

Contaminated fruits are a major vehicle for foodborne illnesses, which remain a leading cause of morbidity in Nigeria, and identifying bacterial pathogens in spoilt fruits will help highlight potential health risks (FAO, 2020; WHO, 2019). Oranges and watermelons, being widely cultivated and consumed in Jigawa State, have significant economic importance, and post-harvest losses not only reduce farmer income but also threaten food security. Scientifically, the study will provide baseline data on the bacterial flora of spoilt fruits in Dutse. The findings also have policy relevance, as they can inform local health authorities, NAFDAC, and agricultural agencies on strategies to improve handling, storage, and market hygiene practices. This study aimed to isolate, identify and determine the antibiotic susceptibility profile of bacterial pathogens associated with spoilt oranges and watermelons sold in Dutse, Jigawa State.

Materials and Methods

Study Area

The study was conducted in Dutse, the capital of Jigawa State, Northwestern Nigeria. Dutse lies on latitude 11°43′N and longitude 9°20′E, at an altitude of approximately 460 m above sea level (NIMET, 2021). Dutse hosts major fruit markets where oranges (*Citrus sinensis*) and watermelons (*Citrullus lanatus*) are sold in large quantities.

Sample Collection

Spoilt oranges and watermelons were collected from selected fruit markets within Dutse metropolis. Samples were chosen based on visible signs of microbial spoilage such as softening, discoloration, watery lesions and foul odor. A total of twenty (20) fruit samples were collected, ten (10) spoilt oranges and ten (10) spoilt watermelons. Each sample was aseptically transferred into sterile polyethylene bags, labeled appropriately, and transported in an ice-packed container to the Botany Laboratory, Faculty of Science, Federal University Dutse, for microbiological analysis.

Isolation of Bacteria from Spoilt Fruits

Preparation of Samples

Approximately 10g of spoilt fruit tissue was aseptically excised from each sample using a sterile scalpel. The tissue was homogenized in 90 ml of sterile distilled water to obtain a stock suspension (10^{-1} dilution). Serial dilutions were prepared up to 10^{-5} according to the method described by Rahman et al. (2022).

Inoculation and Incubation

The pour plate method was employed for bacterial isolation. One milliliter (1 ml) of each serial dilution was aseptically transferred into sterile Petri dishes. Approximately 15 ml of sterile molten Nutrient Agar, cooled to about

45°C, was poured into each dish, gently mixed, and allowed to solidify. Selective plating was also carried out by streaking the samples on specific media to aid bacterial identification. Eosin Methylene Blue (EMB) Agar was used for isolating coliforms such as *Escherichia coli*, MacConkey Agar was used to detect lactose-fermenting enteric bacteria, while *Salmonella-Shigella* (SS) Agar was used for the detection of *Salmonella* and *Shigella* species. All inoculated plates were incubated at 37°C for 24 to 48 hours to allow visible colony growth (Nwachukwu et al., 2018). Distinct colonies obtained after incubation were carefully sub-cultured onto Nutrient Agar slants to obtain pure cultures and preserved for further biochemical and morphological characterization.

Characterization and Identification of Isolates

Microscopic Examination (Gram Staining)

Gram staining was carried out using Crystal violet, Lugol's iodine, 70% alcohol, and Safranin. The stained smears were observed under the oil immersion objective (100×) to determine: Gram reaction (positive or negative) and Cell shape (cocci, bacilli, or rods). The Gram staining result served as the primary classification method for the isolates, distinguishing between Gram-positive and Gram-negative bacteria.

Biochemical Characterization

Biochemical tests were conducted to further identify and characterize the bacterial isolates based on their physiological and metabolic activities. The lactose fermentation test was carried out on MacConkey Agar to determine the ability of the isolates to ferment lactose, with positive results indicated by the production of pink colonies. The Eosin Methylene Blue (EMB) test was performed to detect *Escherichia coli*, where the appearance of a metallic green sheen on the agar surface confirmed its presence. The hydrogen sulfide (H_2S) test was conducted using *Salmonella-Shigella* Agar to identify *Salmonella* species, with the formation of black precipitates indicating a positive result. The catalase test was used to detect the presence of the catalase enzyme by adding hydrogen peroxide to the culture and observing effervescence or bubble formation as a positive reaction (Nwachukwu et al., 2018). Finally, the oxidase test was employed to determine the presence of cytochrome oxidase enzymes, indicated by a rapid color change (usually to purple) upon the addition of the oxidase reagent.

Procedure for Biochemical Tests

Coagulase (CG) Test

According to the method described by Forbes et al. (2023), a sterile normal saline was dropped on a clean glass slide, small amount of fresh bacterial isolate was emulsified in the saline to form a smooth suspension. one drop of plasma was added to the bacterial suspension and mixed gently with a sterile applicator stick; observation was made for visible clumping (agglutination).

Catalase (CT) Test

As described by Centers for Disease Control and Prevention (2024), a clean, dry glass slide was placed on the workbench and 2 drops of 3% hydrogen peroxide (H_2O_2) was added onto the slide. Using a sterile platinum loop, a small amount of bacterial colony was transferred into the hydrogen peroxide drop. Observation was made immediately for the production of bubbles (oxygen gas), which indicates the breakdown of hydrogen peroxide by the enzyme catalase.

Indole (ID) Test

A sterile test tube containing tryptone broth was labeled, followed by inoculating the broth with a pure bacterial isolate using a sterile inoculating loop, the inoculated broth was incubated at 35–37°C for 18–48 hours. After incubation, 0.5 mL of Kovac's reagent was added to the surface of the broth without mixing and the reagent was allowed to stand for 1–2 minutes, then, the color of the reagent layer at the top of the broth was observed (Cappuccino and Welsh, 2024).

Methyl Red (MR) Test

As also described by Forbes et al. (2023), a sterile test tube containing MR-VP broth was labeled,

the broth was inoculated with a pure bacterial isolate using a sterile inoculating loop, the inoculated broth was incubated at 35–37°C for 48 hours. After incubation, 2 mL of the culture was transferred into a clean test and 5 drops of methyl red indicator was directly added to the broth and gently mixed then, the color was observed immediately.

Citrate utilization (CT) Test

A Simmons citrate agar slant was labeled and a small amount of a pure bacterial colony was picked using a sterile inoculating needle, the surface of the citrate agar slant was lightly streaked from the bottom to the top, the inoculated slant was incubated at 35–37°C for 24–48 hours (ASM, 2024). The slant was examined for bacterial growth and any change in the color of the medium.

Urease (UR) Test

A Christensen's urea agar slant was labeled, and fresh pure bacterial colony was picked using a sterile inoculating loop, the surface of the urea agar slant was streaked aseptically, and the inoculated medium was incubated at 35–37°C for 18–24 hours; then, observed the medium for any color change (Cappuccino and Welsh, 2024).

Voges Proskauer (VP) Test

A sterile test tube containing MR-VP broth was labeled followed inoculating the broth with a pure bacterial isolate using a sterile inoculating loop, the inoculated broth was incubated at 35–37°C for 24–48 hours. About 2 mL of the incubated broth was transferred into a clean test tube, and 0.6 mL of Barritt's Reagent A (5% α-naphthol) was added and mixed well. 0.2 mL of Barritt's Reagent B (40% potassium hydroxide, KOH) was also added and shaken vigorously to introduce oxygen, which is required for the reaction, then, allowed the tube to stand for 20 minutes at room temperature and the color development was observed (Forbes et al., 2023).

Susceptibility testing

The Kirby-Bauer disk diffusion method was used to determine the susceptibility of the bacterial isolates to antibiotics. A pure bacterial isolates were evenly spread over the surface of Mueller Hinton agar plates to form a uniform bacterial lawn. Small paper disks impregnated with different concentrations of antibiotics are placed on the agar surface. After incubation, the diameter of each zone of inhibition was measured (mm) and compared with standard interpretive charts.

Data Analysis

Descriptive statistics such as means and standard deviations were used to summarize the data.

Results and Discussion

Result

Table 1 shows the total viable bacterial counts, Gram reactions, and morphological characteristics of isolates collected from Orange and Watermelon fruits. The results display a wide range of microbial contamination. The highest bacterial load was observed on Orange sample S4 with a viable count of 2.15×10^5 CFU/mL, while the lowest was found on Orange sample S6 with 1.3×10^4 CFU/mL.

Both Gram-positive and Gram-negative bacteria were observed. Dominant Gram-positive bacteria included *Staphylococcus spp.* and *Bacillus spp.*, whereas *Pseudomonas spp.* and *Escherichia coli* were the main Gram-negative isolates. The variation in bacterial load suggests differences in surface handling or exposure to unhygienic environments.

Table 2 summarizes the biochemical characteristics of the bacteria isolated from both fruits. In Orange samples, the most common organisms were *Staphylococcus spp.* and *Pseudomonas spp.* (each 33.33 %), followed by *E. coli and Bacillus spp.* (16.67% each). In Watermelon, *Pseudomonas spp.* was most prevalent (37.50 %), with E. coli at 25.00 %, while *Staphylococcus spp., Klebsiella spp.,* and *Bacillus spp.* each accounted for 12.50 %.

Table 3 summarizes the antibiotic susceptibility profiles of the bacterial isolates from Orange and Watermelon. The results highlight that E. *coli* is susceptible to meropenem and chloramphenicol and resistant to nalidixic acid and

sulfamethoxazole, indicating potential misuse pressure by these antibiotics in the hospital. *Staphylococcus spp* exhibited susceptibility to vancomycin, gentamicin, novobiocin, and chloramphenicol while resistant to nalidixic acid and sulfamethoxazole, consistent with widespread antibiotic resistance patterns seen in nosocomial pathogens. *Pseudomonas spp* is susceptible to meropenem and chloramphenicol and resistant to gentamIcin, demonstrating its well-known multidrug-resistant nature.

Table 1: Bacterial count, Gram Reaction and Morphology of Isolates

Fruits	Samples	Total Viable Count (CFU/mL)	Gram Reaction	Genus	Shapes
Orange	S1	2.6×10^4	Gram positive	*Staphylococcus*	Cocci
	S2	3.1×10^4	Gram positive	*Bacilli*	Rod
	S3	2.0×10^4	Gram positive	*Bacilli*	Rod
	S4	2.15×10^5	Gram positive	*Staphylococcus*	Cocci
	S5	4.2×10^4	Gram negative	*Bacilli*	Rod
	S6	1.3×10^4	Gram negative	*Bacilli*	Rod
	S7	1.66×10^5	Gram positive	*Streptococcus*	Cocci
	S8	2.3×10^4	Gram positive	*Streptococcus*	Cocci
	S9	3.8×10^4	Gram negative	*Bacilli*	Rod
	S10	6.5×10^4	Gram negative	*Bacilli*	Rod
Water melon	S11	3.5×10^4	Gram positive	*Staphylococcus*	Cocci
	S12	4.8×10^4	Gram positive	*Streptococcus*	Chain
	S13	5.9×10^4	Gram positive	*Staphylococcus*	Cocci
	S14	6.7×10^4	Gram negative	*Bacilli*	Rod
	S15	4.0×10^4	Gram negative	*Bacilli*	Rod
	S16	2.4×10^4	Gram positive	*Staphylococcus*	Cocci
	S17	1.75×10^5	Gram positive	*Staphylococcus*	Cocci
	S18	6.8×10^4	Gram positive	*Streptococcus*	cocci
	S19	5.7×10^4	Gram negative	*Bacilli*	Rod
	S20	3.2×10^4	Gram positive	*Staphylococcus*	Cocci

Table 2: Biochemical Characteristics of Bacterial Isolates

FT	Biochemical Test							Suspected Organism	Oc	Prev. (%)
	CG	CT	ID	MR	CR	VP	UR			
Or	-	+	+	+	-	-	-	*Escherichia coli*	1	16.67
	+	+	-	+	+	+	+	*Staphylococcus spp*	2	33.33
	-	+	-	-	+	-	-	*Pseudomonas spp*	2	33.33
	+	+	-	-	+	+	-	*Bacillus spp*	1	16.67
	-	-	-	+	-	-	-	*Streptococcus spp*	2	33.33
WM	-	+	+	+	-	-	-	*Escherichia coli*	2	25.00
	+	+	-	+	+	+	+	*Staphylococcus spp*	1	12.50
	+	+	-	-	+	+	+	*Klebsiella spp*	1	12.50
	-	+	-	-	+	-	-	*Pseudomonas spp*	3	37.50
	+	+	-	-	+	+	-	*Bacillus spp*	1	12.50

Keys: CG – Coagulase, CT- Catalase, ID- Indole, MR- Methyl Red, CR= Citrate, VP- Voges Proskaeur, UR- Urease, +/-: Positive/Negative Gram reaction, FT- fruit type, Or-orange, WM- watermelon, Oc- occurrence

Table 3: Antibiotic Susceptibility of Bacterial Isolates

Bacterial Isolate	Mer	Van	Gen	Nal	Sul	Nov	Chlo	Ery
Escherichia coli	S	NA	S	R	R	NA	S	NA
Staphylococcus spp	NA	S	S	R	R	S	S	NA
Pseudomonas spp	S	NA	R	NA	NA	NA	S	NA
Bacillus spp.	R	R	R	R	R	R	R	S
Klebsiella spp	S	NA	NA	R	R	NA	S	NA

Keys: NA= Not applicable, S= Susceptible, R = Resistant, Mer = Meropenem, Van = Vancomycin, Gen = Gentamicin, Nal = Nalidixic Acid, Sul = Sulfamethoxazole, Nov = Novobiocin, Chlo = Chloramphenicol, Ery = Erythromycin

Bacillus spp. is susceptible only to erythromycin, highlighting its distinct antibiotic sensitivity compared to other isolates. *Klebsiella spp* isolates were susceptible to meropenem and chloramphenicol and resistant to nalidixic acid and sulfamethoxazole, mirroring resistance patterns observed in *E. coli*.

Discussion

The findings of this study provide a critical insight into the microbiological quality of commonly consumed fresh fruits oranges and watermelons highlighting their potential role as vehicles for the transmission of pathogenic and antibiotic-resistant bacteria. The isolation of both Gram-positive and Gram-negative bacteria, including *Staphylococcus spp.*, *Streptococcus spp.*, *Escherichia coli*, *Pseudomonas spp.*, *Klebsiella spp.*, and *Bacillus spp.*, suggests that these fruits may serve as reservoirs for opportunistic or enteric pathogens, particularly when handled or stored under unhygienic conditions.

The high total viable counts recorded on both fruits, especially the 2.15×10^5 *CFU/mL* observed on orange sample S4, exceed the acceptable microbial limits for ready-to-eat fresh produce. According to the International Commission on Microbiological Specifications for Foods (ICMSF, 2002), counts above 10^4 CFU/mL indicate a potential public health hazard, especially when such fruits are consumed raw. This aligns with reports by Rahman et al. (2022), who documented high microbial loads in fruits sold in open markets and linked them to improper post-harvest handling and environmental exposure.

The predominance of *Staphylococcus spp.* and *Pseudomonas spp.* in orange samples suggests contamination through frequent human contact and environmental sources, respectively. *Staphylococcus spp.* is commonly found on human skin and nasal passages and is often introduced into foods through improper handling (Olaimat & Holley, 2021; Kim & Cheigh, 2021). Its presence on fruit surfaces reflects lapses in personal hygiene among handlers. *Pseudomonas spp.*, a known spoilage organism, thrives in moist environments and is commonly associated with water and soil contamination, indicating that unclean washing water or unsanitized surfaces could have contributed to contamination (Murray et al., 2019).

The high prevalence of *Escherichia coli* and *Pseudomonas spp.* in watermelon samples underscores the risk of fecal and environmental contamination. *E. coli* is an indicator organism for fecal pollution and poor sanitation. Its detection supports findings from similar studies where fruits were irrigated with contaminated water or handled with unwashed hands (Liu et al., 2015). The identification of *Klebsiella spp.* further strengthens concerns about enteric bacteria presence and potential health risks, especially for immunocompromised individuals.

Antibiotic susceptibility testing revealed alarming levels of resistance among isolates. *Escherichia coli* and *Klebsiella spp.* were resistant to nalidixic acid and sulfamethoxazole, drugs commonly used in treating urinary and gastrointestinal infections. This may indicate

environmental exposure to antibiotics or the selective pressure from excessive use of antimicrobials in agriculture and animal husbandry (WHO, 2020). The emergence of antibiotic-resistant bacteria on fresh produce is a global concern, as highlighted by Abadias et al., (2020) and Rahman et al., (2022) who reported that resistance genes in environmental bacteria can be transferred to human pathogens via the food chain. The findings further stress the need for food safety monitoring, especially in developing countries where food hygiene regulations are often poorly enforced. Finally, the study reflects a broader public health challenge: the intersection between foodborne contamination and the rise of antimicrobial resistance (AMR). The World Health Organization (2020) identifies AMR as one of the top ten global health threats, and this study contributes to the evidence that food, particularly fresh produce, may act as a tube for the spread of resistant organisms from the environment to humans.

Conclusion

This study demonstrates that fruits such as oranges and watermelons can harbor a variety of potentially pathogenic bacteria, including multidrug-resistant strains. The high microbial loads and presence of resistant organisms reflect poor post-harvest handling practices and inadequate hygiene during storage and sale. These findings present significant public health concerns, especially in communities where fruits are consumed without proper washing or peeling.

Recommendations

Strict hygiene protocols should be enforced during fruit harvesting, transportation, and retailing to reduce microbial contamination. Public health education campaigns should inform vendors and consumers about the importance of washing fruits thoroughly before consumption. Finally, routine microbial surveillance of fresh produce should be implemented by food safety authorities to monitor contamination levels and detect resistant strains early.

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