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Molecular identification and antibiotic susceptibility of bacteria from watermelon in southwestern Nigeria

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Abstract. *Akindele ST, Agbolade OA, Osiyemi EO, Raufu TT, Obafebi TO, Olukoga MT, Adetokunbo KS, Olatunji MO. 2025. Molecular identification and antibiotic susceptibility of bacteria from watermelon in southwestern Nigeria. Asian J Trop Biotechnol 22: 64-70.* Watermelon (*Citrullus lanatus*) is a widely consumed fruit in Nigeria, often eaten fresh without further processing. However, its ready-to-eat nature predisposes it to microbial contamination and potential transmission of antibiotic-resistant bacteria. This study applied integrated microbiological and molecular approaches to characterize bacterial communities associated with sliced watermelon and assess their antimicrobial susceptibility under tropical conditions. Fresh watermelon samples were collected from Oru (Ogun State) and Iseyin (Oyo State), Nigeria. Standard microbiological methods were employed to determine total viable counts, bacterial counts, and lactic acid bacteria counts. Isolates were characterized using biochemical tests, 16S rRNA gene sequencing, and phylogenetic analysis. Antimicrobial susceptibility was assessed using the disc diffusion method against commonly used antibiotics. Microbial analysis revealed higher bacterial loads in Iseyin samples than in Oru. Seven bacterial species were identified, including *Lactobacillus reuteri, Bacillus mendelii, Curtobacterium flaccumfaciens, Lactobacillus helveticus, Acinetobacter lwoffii, Lactobacillus casei,* and *Lactobacillus vaccinostercus*. Phylogenetic analysis confirmed close evolutionary relatedness with reference strains. Most isolates displayed high resistance to pefloxacin and amoxicillin but were sensitive to ceftriaxone and chloramphenicol. The dominance of *Bacillus* species and detection of lactic acid bacteria highlighted both spoilage and potential probiotic roles. This study provides baseline data for molecular surveillance of antimicrobial resistance in tropical environments and demonstrates how molecular identification and AST can inform food biotechnology, probiotic exploration, and safety management of fresh produce.

Keywords: Antibiotics, antimicrobial susceptibility, *Citrullus lanatus*, molecular techniques, watermelon

INTRODUCTION

Fresh fruits are widely recognized for their nutritional content, sensory appeal, and contribution to a healthy diet. Among these, watermelon (*Citrullus lanatus*), a member of the Cucurbitaceae family, is valued for its sweetness, juiciness, and hydrating properties. It is cultivated extensively in warm climates worldwide and is increasingly consumed for its nutritional and health promoting benefits (Harith et al. 2018; Sodimu et al. 2020). However, the safety of fresh produce has become a global public health concern, as microbial contamination can occur at multiple stages of production including cultivation, harvesting, transportation, storage, and retail display (Balali et al. 2020; Sane et al. 2024). Such contamination compromises fruit quality and may serve as a vehicle for foodborne pathogens, thereby posing a potential risk to consumers (Rahman et al. 2022).

Watermelon, like other ready-to-eat fruits, is highly susceptible to microbial colonization by both spoilage and pathogenic organisms. The increased demand for fresh fruits and vegetables has amplified this risks, as these commodities are typically consumed raw or with minimal processing, allowing microorganisms to persist. Understanding the microbial diversity of watermelon is therefore essential in food microbiology and food biotechnology, particularly for improving postharvest handling and developing microbial-based bio-preservation strategies (Alegbeleye et al. 2018).

Traditional culture-based methods remain useful in microbial studies but are limited by their inability to capture the complete microbial diversity, especially for fastidious or non-culturable species. Advances in molecular biotechnology, particularly 16S rRNA gene sequencing have transformed microbial ecology by providing rapid, precise, and culture-independent identification (Ilyanie et al. 2023; Hadi et al. 2023; Alessandro et al. 2024; Gomes et al. 2025). These molecular tools not only improve taxonomic resolution but also facilitate phylogenetic analysis and evolutionary interpretation of microbial isolates, making them indispensable for modern biotechnological applications in food safety, environmental monitoring, and probiotic development.

Equally critical is the growing global threat of Antimicrobial Resistance (AMR), which undermines both clinical therapy and food security (Pachillu et al. 2024). Fresh produce has been increasingly recognized as a potential reservoir for antibiotic-resistant bacteria, raising concerns about the dissemination of resistance determinants through the food chain (Klaui et al. 2024).

The use of Antimicrobial Susceptibility Testing (AST) in food biotechnology therefore extends beyond clinical diagnostics, it supports surveillance, risk assessment, and development of antimicrobial alternatives for bio-preservation (Kalpana et al. 2024). Integrating AST with molecular identification can reveal resistance patterns linked to specific bacterial taxa and provide insights into resistance ecology within food matrices.

Despite the global significance of AMR surveillance, limited information exists on the microbial composition and resistance profiles of bacteria isolated from watermelon in Nigeria. Most previous studies on fresh produce have focused on microbial load estimation or morphological identification of spoilage fungi, without incorporating molecular sequencing and antibiotic susceptibility profiling. Consequently, the role of watermelon as a potential carrier of antibiotic-resistant bacteria remains poorly understood, particularly within tropical environments where high humidity and temperature favor microbial proliferation.

This study was therefore designed to isolate and characterize bacteria associated with sliced watermelon using both phenotypic and molecular methods, and to evaluate their antibiotic susceptibility patterns. By combining 16S rRNA gene sequencing with AST, the study provides a molecular-level understanding of microbial diversity and resistance in watermelon. In addition to identifying potential food safety risks, the research highlights how molecular biotechnology can inform microbial surveillance, probiotic screening, and bio-preservation strategies in tropical fruit systems.

Unlike earlier reports that relied primarily on culture-based identification or descriptive contamination data, this work integrates molecular and biotechnological perspectives to generate comprehensive baseline information for foodborne bacterial ecology in Nigeria. The findings are expected to contribute to food biotechnology by supporting the development of molecular monitoring systems, antimicrobial stewardship, and sustainable safety interventions for fresh produce in tropical regions.

MATERIALS AND METHODS

Sample collection and preparation

A total of ten (10) samples (five each) of sliced watermelon fruits (*C. lanatus*) were collected from Oru Expressway, Ogun State, and Iseyin Market, Oyo State, Nigeria respectively. The samples were randomly obtained during rainy season and were collected from different vendors to minimize false replication and enhance representativeness. The samples were held with sterile gloves and were aseptically transferred to the laboratory in sterile containers for analysis in an insulated ice pack. Each sample (2 g) was homogenized using a sterile mortar and pestle, and 1 g of homogenate was suspended in 9 mL of sterile distilled water in a McCartney bottle to obtain the initial dilution. Serial tenfold dilutions were prepared, and 0.1 mL aliquots of 10^{-3}, 10^{-5}, and 10^{-6} dilutions were plated in triplicate using the pour plate technique on Nutrient Agar (NA), *Lactobacillus*-MRS Agar, and Mueller- hinton agar (MHA). Plates were incubated at 37°C for 24 hours, after which colonies were enumerated and purified for further characterization (Balali et al. 2020). Sterile control media plates were also incubated to monitor laboratory sterility.

Biochemical and molecular identification

Purified isolates were characterized by standard biochemical tests, including catalase, oxidase, coagulase, methyl red, indole, citrate utilization, and sugar fermentation, following the method of Anka et al. (2023). Genomic DNA was extracted using the Quick-DNA™ Miniprep Plus Kit (Zymo Research, USA) according to the manufacturer's instructions. Extracted DNA was quantified using a NanoDrop spectrophotometer, and quality was verified by 1.5% agarose gel electrophoresis.

The 16S rRNA gene was amplified using universal primers (27F and 1492R), and PCR products were visualized on 1.5% agarose gel under UV transillumination. Amplicons were purified and sequenced commercially. Sequence chromatograms were trimmed and analyzed for quality control using MEGA X and BioEdit software. High-quality sequences were compared with reference sequences in the NCBI GenBank database using BLAST, and representative sequences were deposited in GenBank. Phylogenetic analysis was conducted using the neighbor-joining method with 1,000 bootstrap replications.

Antibacterial susceptibility testing

Antimicrobial susceptibility of isolates was evaluated using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar, following Clinical and Laboratory Standards Institute (CLSI 2021) guidelines. The bacterial inocula were standardized (0.5 McFarland turbidity) and were then spread on Muller-Hinton agar. Thereafter the antibiotic discs were applied. Thirteen antibiotic discs (Oxoid, UK) were used: ceftriaxone (30 µg), ampiclox (10 µg), cefuroxime (30 µg), chloramphenicol (30 µg), spiramycin (100 µg), ofloxacin (5 µg), erythromycin (15 µg), streptomycin (10 µg), cotrimoxazole (25 µg), amoxicillin (25 µg), ciprofloxacin (5 µg), gentamicin (10 µg), and pefloxacin (5 µg). The plates were the incubated at 37^{O}C for 24 hours and zones of inhibition were measured in millimeters, and results were interpreted as sensitive, intermediate, or resistant according to CLSI (2021) breakpoints. No reference control strain was available. However, the sterility control ensured validity of the results.

Statistical analysis

Data were analyzed using IBM SPSS Statistics version 17.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize microbial counts, while inferential analysis was performed using binary logistic regression to assess factors associated with antibiotic resistance. The Wald chi-square test was applied to evaluate significant differences at a 95% confidence level ($p<0.05$).

RESULTS AND DISCUSSION

Microbial counts

Microbial analysis of fresh watermelon samples showed significant variation in microbial load between the two locations (Table 1). The Total Viable Count (TVC) in samples from Iseyin (8.18×10^6 cfu/g) was markedly higher ($p<0.05$) than in samples from Oru (2.01×10^3 cfu/g). Similarly, bacterial counts were substantially greater in Iseyin (6.73×10^6 cfu/g) compared with Oru (1.0×10^1 cfu/g), indicating heavier contamination. Lactic acid bacteria (LAB) counts were also higher in Iseyin (8.18×10^3 cfu/g), suggesting active microbial metabolism. The elevated microbial burden in Iseyin likely reflects environmental exposure, water quality, and poor post-harvest handling practices typical of open-market conditions, whereas the comparatively lower counts in Oru suggest better sanitary control and handling practices.

Biochemical and molecular characterization

Biochemical characterization of isolates (Table 2) revealed considerable metabolic diversity, with all isolates being Gram-positive rods showing variable catalase and oxidase reactions. Fermentation of simple sugars such as glucose, fructose, and sucrose differentiated the isolates, suggesting the presence of multiple genera with distinct metabolic pathways.

PCR amplification of the 16S rRNA gene from all isolates (AAP1–AAP7) yielded clear amplicons of approximately 250 bp (Figure 1). Successful amplification across all isolates confirmed the efficiency of the primers and the quality of extracted DNA. The 16S rRNA sequences obtained were aligned and compared with GenBank references, revealing seven bacterial species: *Lactobacillus reuteri*, *Bacillus mendelii*, *Curtobacterium flaccumfaciens*, *Lactobacillus helveticus*, *Acinetobacter lwoffii*, *Lactobacillus casei*, and *Lactobacillus vaccinostercus*. Phylogenetic analysis (Figure 2) demonstrated strong bootstrap support for clustering with reference strains, confirming accurate taxonomic identification.

Integrating the biochemical and molecular data revealed good concordance between phenotypic characteristics and sequence-based classification. For example, *Lactobacillus* species exhibited typical Gram-positive, catalase-negative, acid-producing reactions, consistent with their molecular identities. In contrast, the *Bacillus* isolate displayed catalase-positive and motile traits, aligning with its phylogenetic placement. This integration highlights the complementary value of combining classical and molecular tools in microbial biotechnology.

Antimicrobial susceptibility profiles

The antimicrobial susceptibility patterns of the isolates are presented in Table 3. Resistance levels varied across antibiotics, with the lowest resistance observed against ceftriaxone, ampiclox, cefuroxime, chloramphenicol, and spiramycin (<30%). Moderate resistance (40-60%) was recorded for ofloxacin, erythromycin, streptomycin, and cotrimoxazole, while high resistance (>70%) was detected against amoxicillin, ciprofloxacin, and gentamicin. Notably, all isolates (100%) were resistant to pefloxacin.

When analyzed statistically, resistance frequencies differed significantly among antibiotic classes ($p<0.05$), with fluoroquinolones and aminoglycosides showing the highest resistance proportions. The emergence of multidrug-resistant isolates underscores the potential of watermelon as a reservoir for resistant bacteria. However, sensitivity to chloramphenicol and ceftriaxone suggests that some conventional antibiotics may still retain therapeutic potential.

Table 1. Microbial counts (cfu/g) of fresh watermelon samples collected from Oru and Iseyin, Nigeria

Sample codes	Total viable counts (cfu/g)	Bacteria counts (cfu/g)	Lactic acid bacteria counts (cfu/g)
Oru fresh watermelon	2.01×10^3	1.0×10^1	1.0×10^1
Iseyin fresh watermelon	8.18×10^6	6.73×10^6	8.18×10^3

Table 2. Biochemical test results of bacteria isolated from sliced watermelon samples

Isolate ID	Catalase	Oxidase	Glucose	Fructose	Lactose	Sucrose	Sulphate reduction	Indole	Motility	Gram reaction	Gram shape
1	+	+	Acid + Gas	Acid	No Change	No Change	-	-	-	+	Rod
2	-	-	No Change	No Change	No Change	Acid	-	-	-	+	Rod
3	-	+	Acid	Acid	Acid	Acid	+	-	-	+	Rod
4	+	+	Acid	Acid	No Change	Acid	+	-	-	+	Rod
5	-	-	Acid	Acid	Acid	Acid	-	-	+	-	Rod
6	+	-	No Change	No Change	No Change	No Change	-	-	+	+	Rod
7	+	-	No Change	No Change	No Change	No Change	-	-	-	+	Rod

Note: +: Positive, -: Negative

Table 3. Mean zones of inhibition of bacterial isolates against tested antibiotics according to CLSI interpretive standards

Antibiotic	Resistance (%)	Mean zone of inhibition (mm)	CLSI interpretation
Rocephin	15	23.6±2.1	Susceptible (S)
Ampiclox	28	21.5±1.9	Susceptible (S)
Zinnacef	30	21.2±1.8	Susceptible (S)
Chloramphenicol	30	21.2±1.8	Susceptible (S)
Spiramycin	30	21.2±1.8	Susceptible (S)
Oflaxacin	45	18.8±2.0	Intermediate (I)
Erythromycin	52	17.7±1.9	Intermediate (I)
Streptomycin	55	17.2±2.1	Intermediate (I)
Septrin	60	16.4±1.7	Intermediate (I)
Amoxicillin	62	16.1±1.8	Intermediate (I)
Ciprofloxacin	70	14.8±1.9	Resistant (R)
Gentamycin	90	11.6±2.0	Resistant (R)
Perfloxacin	100	10.0±1.5	Resistant (R)

Note: S≥21 mm, I=16-20 mm, R≤15 mm

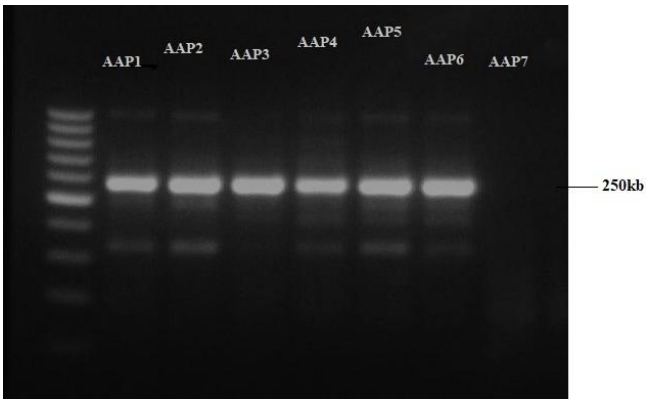

Figure 1. Gel electrophoresis of 16S rRNA gene amplicons from bacterial isolates obtained from sliced watermelon samples

Discussion

This study investigated the microbial quality, molecular identification, and antibiotic susceptibility of bacteria isolated from sliced watermelon sold in Oru (Ogun State) and Iseyin (Oyo State), Southwestern Nigeria. The results revealed marked variation in microbial loads, with the Iseyin samples exhibiting significantly higher counts compared to Oru. These differences likely stem from varying levels of environmental hygiene, water quality, and fruit handling practices at the points of sale. Balali et al. (2020) similarly reported that post-harvest handling and market sanitation strongly influence microbial contamination levels in fresh produce. In the context of biotechnology, understanding these contamination dynamics is critical for developing microbial-based safety interventions and environmental monitoring systems for tropical foods.

Biochemical and molecular characterization revealed a mixture of beneficial and opportunistic bacterial species. The presence of Lactic Acid Bacteria (LAB), including *L. reuteri*, *L. casei*, and *L. helveticus*, indicates a natural fermentation potential that could be harnessed for bio-preservation and probiotic development. LAB are known to produce bacteriocins and organic acids capable of inhibiting spoilage and pathogenic microorganisms, making them useful candidates in applied food biotechnology (Alegbeleye et al. 2018; Harith et al. 2018). The detection of these species on watermelon surfaces suggests that they may play a protective ecological role and could be explored as starter cultures in functional fruit-based fermentations.

Conversely, the recovery of *A. lwoffii* and *B. mendelii* points to environmental and opportunistic contamination. *A. lwoffii* is commonly associated with soil, water, and human contact surfaces and has been implicated in nosocomial infections (Rajkumari et al. 2020). Its presence on ready-to-eat watermelon slices underscores the risk of cross-contamination during handling and vending. *Bacillus* species, while ubiquitous, can form resistant endospores that survive adverse conditions and contribute to food spoilage. These findings highlight the microbial complexity of fresh produce and the need for biotechnological monitoring systems that integrate rapid molecular detection with microbial risk assessment.

The phylogenetic analysis based on 16S rRNA gene sequencing confirmed high sequence similarity between the isolates and reference strains in GenBank, supporting the accuracy and reproducibility of molecular identification. The clustering of *Lactobacillus* and *Bacillus* species within well-supported clades demonstrates the value of 16S rRNA analysis in resolving taxonomic ambiguities that may arise from conventional biochemical tests. This aligns with previous reports by Ilyanie et al. (2023) and Gomes et al. (2025), which emphasized that molecular tools enhance the resolution of microbial diversity studies, particularly in complex food matrices. Beyond taxonomic identification, these molecular datasets also provide opportunities for comparative genomics, strain improvement, and phylogenetic tracing in food biotechnology research.

Antimicrobial susceptibility testing revealed heterogeneous resistance patterns across isolates, with widespread resistance to commonly used antibiotics such as amoxicillin, ciprofloxacin, and gentamicin. The 100% resistance observed against pefloxacin, a fluoroquinolone, is of particular concern and suggests the circulation of Multidrug-Resistant (MDR) bacteria within the food environment. Similar findings have been documented in other food commodities, including vegetables and seafood, indicating the persistence of antimicrobial resistance across different ecological niches (Odeyemi 2016; Stanley et al. 2022; Habib et al. 2023; Kalpana et al. 2024; Klaui et al. 2024; Castello et al. 2025). The persistence of MDR strains in fresh produce presents challenges not only to food safety but also to public health, as resistance determinants may be transferred horizontally to other bacteria (Elsafi et al. 2024). From a biotechnology perspective, such isolates can serve as useful models for studying resistance gene dynamics and for developing molecular biosensors for AMR surveillance (Billington et al. 2022; Alessandro et al. 2024; Wang et al. 2025).

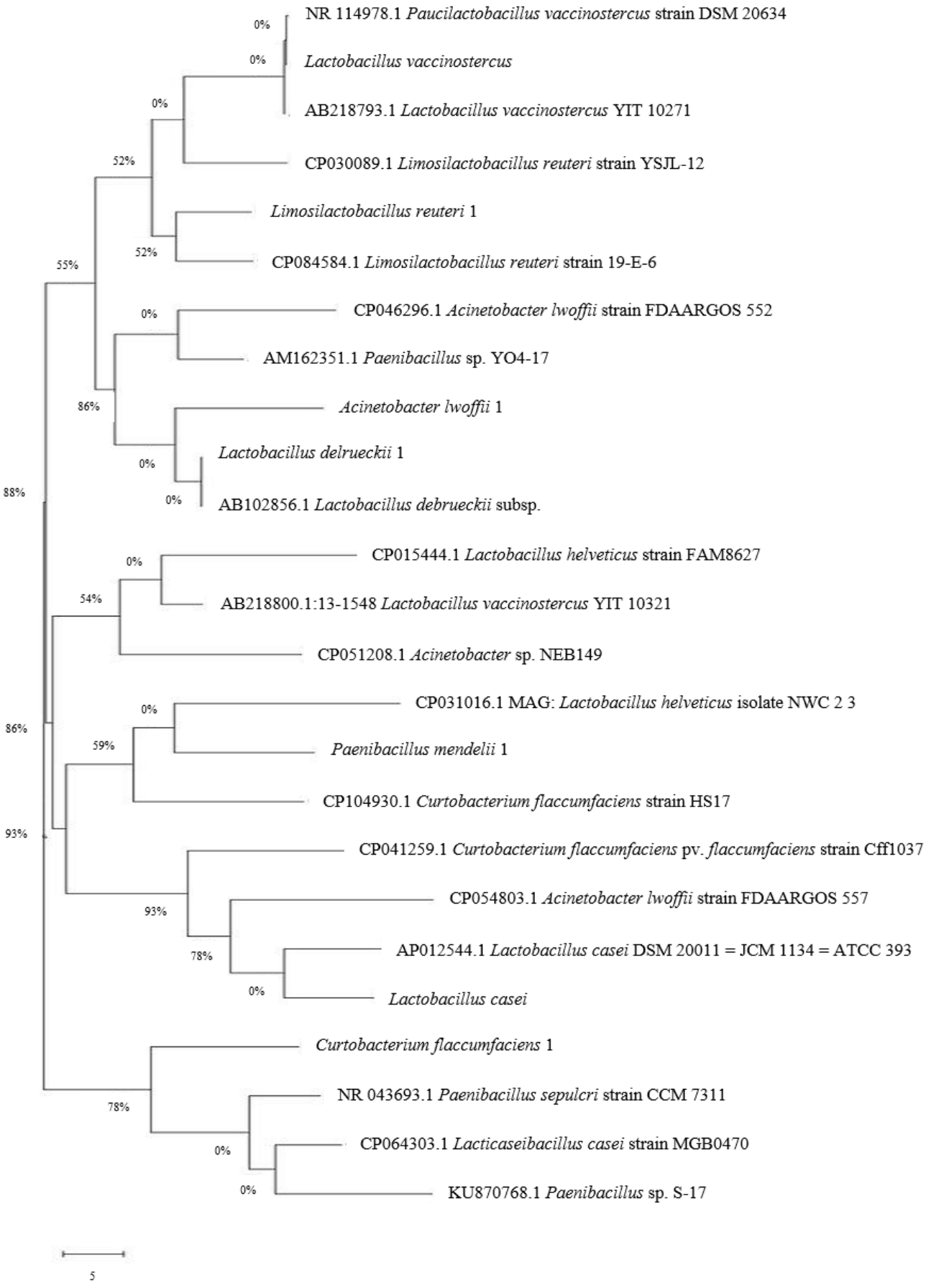

Figure 2. Phylogenetic tree based on 16S rRNA gene sequences of bacterial isolates from sliced watermelon samples

Despite these resistance concerns, several isolates remained sensitive to ceftriaxone and chloramphenicol, suggesting that some therapeutic options remain viable. The variability in susceptibility profiles may reflect differences in the intrinsic resistance mechanisms and environmental antibiotic exposure of the isolates (Olanbiwoninu et al. 2024; Kiplimo et al. 2025). These observations underscore the importance of integrating Antimicrobial Sensitivity Testing (AST) into routine microbial monitoring and highlight how biotechnology-driven molecular surveillance can complement conventional food hygiene measures.

Collectively, the findings of this study highlight the dual role of watermelon as both a source of beneficial LAB with potential probiotic and bio-preservative applications, and as a vehicle for pathogenic or antibiotic-resistant bacteria. This duality underscores the need for balanced intervention strategies that promote beneficial microbiota while mitigating health risks associated with resistant strains. Similar dual outcomes were reported by Lopez-Galvez et al. (2020), who observed both probiotic and pathogenic microbial populations on fresh-cut fruits.

The integration of molecular identification with antimicrobial susceptibility testing provides a holistic framework for microbial quality assessment in tropical fruit systems. The approach applied in this study advances tropical biotechnology by demonstrating how molecular tools can be applied for microbial surveillance, probiotic screening, and antimicrobial resistance tracking. Strengthening food biotechnology capacities in Nigeria and similar regions through such integrative studies will enhance food safety monitoring, support local bio-preservation innovations, and inform evidence-based policies for controlling antimicrobial resistance in the food chain.

In conclusion, a total of seven bacterial species were identified from sliced watermelon sold in Oru (Ogun State) and Iseyin (Oyo State), Nigeria, with *Bacillus* species being the most dominant group. Phylogenetic analysis based on 16S rRNA gene sequencing confirmed accurate species identification and revealed close evolutionary relationships with known reference strains. Antimicrobial susceptibility testing showed variable resistance patterns, including multidrug resistance to commonly used antibiotics such as pefloxacin and amoxicillin, suggesting that fresh watermelon can serve as a potential reservoir of antibiotic-resistant bacteria. The detection of both beneficial lactic acid bacteria and opportunistic pathogens highlights the dual microbial potential of watermelon, serving both as a source of probiotic organisms and a possible carrier of resistant or pathogenic strains. This duality reflects the complex microbial ecology of tropical fruits and provides opportunities for applied biotechnology, including the screening of indigenous *Lactobacillus* strains for probiotic or bio-preservative use. Beyond its food safety implications, this study demonstrates the relevance of integrating molecular identification and antimicrobial susceptibility testing as biotechnological tools for microbial surveillance, probiotic exploration, and antimicrobial resistance monitoring in fresh produce. Strengthening these molecular approaches in tropical food systems will enhance bio-innovation, improve food quality management, and contribute to sustainable public health strategies.

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