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Isolation, molecular profiling and antibiotics resistance of bacterial pathogens from street-vended soymilk

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Abstract

Street-vended soymilk, derived from Glycine max, is widely consumed for its nutritional value and affordability. However, its high moisture content and nutrient richness make it susceptible to microbial contamination, especially when produced and sold under unhygienic conditions. This study aimed to isolate, molecularly profile, and determine the antibiotic resistance patterns of bacterial pathogens present in street-vended soymilk within a university community. Fresh soymilk samples were aseptically collected from multiple vendors and analyzed using standard microbiological methods. Isolates were characterized phenotypically through colony morphology, Gram staining, lactophenol cotton blue staining, and biochemical tests. Molecular identification was performed via DNA extraction, PCR, amplification of the 16S rRNA gene, agarose gel electrophoresis, and sequencing. Antibiotic Susceptibility Testing of the bacterial isolates were carried out using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar. The zones of inhibition was measured using a meter rule and interpreted according to the guidelines of Clinical and Laboratory Standard Institute (CLSI). The results showed high bacterial counts across the assayed samples (≤4.8 × 10^7CFU/mL). The bacterial isolates were identified as *Salmonella typhi* (98.4% sequence similarity) and *Bacillus cereus* (90.27% similarity). No fungal contamination was detected in any of the samples analyzed. Antibiotic susceptibility testing revealed that *S. typhi* SMIII was susceptible to ciprofloxacin, ceftriaxone, and chloramphenicol but resistant to amoxicillin, while *B. cereus* SMI was susceptible to gentamicin, vancomycin, and erythromycin but resistant to penicillin. Intermediate responses to tetracycline were observed in both isolates. These results highlight the presence of pathogenic and antibiotic-resistant bacteria in street-vended soymilk within the university community, posing potential public health risks. The findings underscore the importance of improved hygiene during processing and handling, routine microbial monitoring, and enforcing public health measures to minimize contamination to ensure consumer safety.

Keywords: Soymilk; Microbial quality; *Bacillus cereus* SMI; *Salmonella typhi* SMIII; Molecular profiling; Antibiotic resistance

1. Introduction

Soymilk, a plant-based beverage derived from Glycine max (soybean), has gained popularity due to its high protein content, essential amino acids, vitamins, and bioactive compounds. It serves as an affordable alternative to dairy milk, especially in developing countries where access to animal protein may be limited [1]. Despite its nutritional benefits, soymilk is highly perishable due to its high water activity and nutrient content, making it prone to microbial contamination [2].

Street-vended soymilk is commonly consumed in urban and semi-urban areas due to its convenience and low cost. However, the production and distribution of these beverages often occur under substandard hygienic conditions, including the use of contaminated water, poor handling practices, and inadequate storage [3]. These factors increase

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the risk of contamination by pathogenic microorganisms, such as *Salmonella* species and *Bacillus cereus*, which can cause foodborne illness.

Salmonella typhi is a Gram-negative bacterium responsible for typhoid fever, a serious systemic infection transmitted through contaminated food and water [4]. *Bacillus cereus*, a Gram-positive spore-forming bacterium, can produce toxins leading to emetic or diarrheal syndromes [5]. Traditional phenotypic and biochemical methods are commonly used to identify these bacteria, but they may lack specificity. Molecular techniques, especially 16S rRNA gene sequencing, allow precise identification and differentiation of bacterial species [6].

With the increasing concern over antimicrobial resistance, evaluating the antibiotic susceptibility of foodborne pathogens is critical. Misuse of antibiotics has led to resistant strains, which can be transmitted via contaminated food [7]. Assessing resistance patterns in bacterial isolates from street foods provides important information for public health interventions.

Despite the growing consumption of street-vended soymilk and its associated public health concerns, there remains limited data on the combined use of conventional microbiological techniques, molecular identification, and antibiotic susceptibility profiling of bacterial contaminants in such products within university environments in developing countries. Previous studies have largely focused on either microbial contamination or antibiotic resistance independently, with few integrating both approaches for comprehensive characterization of isolates. Furthermore, there is a scarcity of localized data on the prevalence and resistance patterns of *Salmonella typhi* and *Bacillus cereus* in street-vended soymilk, particularly within Nigerian university communities. This study therefore bridges this gap by providing a holistic assessment of microbial quality, molecular identity, and antibiotic resistance of bacterial pathogens from street-vended soymilk, thereby contributing valuable data for food safety regulation and public health interventions.

2. Materials and Methods

2.1. Sample Collection

Fresh soymilk samples were purchased from street vendors operating within Enugu State University of Science and Technology (ESUT). Approximately 50 mL of each sample was collected aseptically in sterile containers, kept on ice and transported to the laboratory within 2 h for analysis.

2.2. Isolation of Microorganisms from the soymilk samples.

Aliquots (100 µL) of soymilk were plated on Potato Dextrose Agar (PDA), MacConkey Agar media, Salmonella Shigella Agar media, Deman Rogosa and Sharpe media, Nutrient Agar media and incubated at 37^0C for 24 - 48 h except for PDA which was incubated at 30^0C for 7 days. Colony-forming units per milliliter (CFU/mL) were calculated, and distinct colonies were selected for phenotypic and molecular characterization [8].

2.3. Identification of the Isolates

2.3.1. Phenotypic Characterization

Colonies were examined for morphology including size, shape, color, margin and elevation and were Gram-stained. Biochemical tests, including catalase, oxidase, and methyl red, were performed to support preliminary bacterial identification [9].

2.3.2. Molecular Characterization

Isolation of Genomic DNA from Bacterial Cultures

Genomic DNA was extracted using the ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo Research, USA). Two milliliters of bacterial broth were added to a ZR Bashing™ Lysis Tube with 750 µL lysis solution, homogenized at maximum speed for 5 minutes, and centrifuged at 10,000 × g for 1 minute. Supernatant (400 µL) was transferred to a Zymo-Spin™ IV Spin Filter, centrifuged at 7,000 × g for 1 minute, mixed with 1,200 µL DNA Binding Buffer, and applied to a Zymo-Spin™ IIC Column. Columns were washed with DNA Pre-Wash Buffer and DNA Wash Buffer, and DNA was eluted in 100 µL DNA Elution Buffer.

Assessment of DNA Integrity and PCR Products

DNA quality and PCR amplicons were assessed via agarose gel electrophoresis. Gels (1% for genomic DNA, 2% for PCR products) were prepared in 1× TAE buffer and stained with EZ-Vision™ DNA stain. Samples were electrophoresed and visualized under UV illumination.

Targeted Amplification of 16S rRNA

The 16S rRNA gene was amplified using universal primers:

27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1525R (5′-AAGGAGGTGWTCCARCCGCA-3′). PCR reactions (25 µL) contained 12.5 µL Taq 2× Master Mix, 1 µL of each primer (10 µM), 2 µL DNA template, and 8.5 µL nuclease-free water. Thermal cycling: initial denaturation at 94°C for 5 min; 36 cycles of 94°C for 30 sec, 56°C for 30 sec, 72°C for 45 sec; final extension at 72°C for 7 min; hold at 10°C.

Sequencing and Phylogenetic Analysis of Bacterial DNA:

PCR amplicons were sequenced using the Applied Biosystems 3130xl Genetic Analyzer with the BigDye™ Terminator v3.1 Kit. Sequences were edited in BioEdit and aligned in MEGA X. Bacterial identities were confirmed via BLAST against GenBank sequences, and phylogenetic relationships were inferred.

2.4. Antibiotic Susceptibility Testing

The Kirby-Bauer disk diffusion method on Mueller–Hinton agar was used for the antibiotics susceptibility testing [10]. *S. typhi* SMIII was tested against ciprofloxacin, ceftriaxone, chloramphenicol, tetracycline, and amoxicillin. *B. cereus* SMI was tested against gentamicin, vancomycin, erythromycin, tetracycline, and penicillin. Zones of inhibition were measured using a meter rule and interpreted according to the guidelines of Clinical and Laboratory Standard Institute (CLSI).

3. Results and discussion

3.1. Identification of the Isolates

This study revealed the presence of *Salmonella* and *Bacillus* species in the assayed street-vended soymilk samples. Molecular characterization of the isolates using 16S rRNA gene sequencing confirmed their identities as *S. typh*i and *B. cereus*, supporting earlier phenotypic results. The 16S rRNA gene is a reliable molecular marker for bacterial identification due to its conserved and variable regions. Successful PCR amplification yielded distinct bands at approximately 1.5 kbp, consistent with expected 16S rRNA gene lengths, while the DNA ladder served as an accurate molecular weight reference.

Phylogenetic analysis demonstrated high similarity between the *B. cereus* isolate (SMI) and reference strain 12GK, as well as between *S. typhi* (SMII) and reference strain NCTC 5760, indicating close evolutionary relatedness and confirming species-level identification. Comparable studies have reported similar phylogenetic relationships within the *B. cereus* and *S. typhi* groups [11, 12].

The sequence similarity of 90.27% observed in the *B. cereus* SMI, although slightly below the conventional species-level threshold (~97%), may reflect strain variation or regional genetic divergence among environmental *B. cereus* strains. In contrast, *S. typhi* SMIII exhibited 98.4% sequence similarity with its reference strain, confirming close genetic relatedness.

Elevated bacterial counts across the samples (≤4.8×10^7CFU/mL) suggest substandard hygienic conditions during production, handling, or storage. Similar findings were reported by [13] who observed a high bacterial count of $5.3x10^5$ CFU/mL in soymilk sample sold in Goldie market in Calabar Metropolis

The isolation of *B. cereus* aligns with previous studies reporting its frequent occurrence in protein-rich foods, including plant-based beverages and dairy alternatives [14, 15]. As a spore-forming bacterium, *B. cereus* can survive harsh environmental conditions, resist heat treatment, and germinate from spores under favorable conditions. Its presence in street-vended soymilk likely reflects insufficient pasteurization or cross-contamination during handling.

The detection of *S. typhi* is particularly concerning, given its role as a causative agent of typhoid fever. Its presence indicates potential fecal contamination, possibly arising from unclean water, contaminated raw materials, or lapses in personal hygiene during handling [3, 4]. Similar studies on street-vended foods in developing countries have also reported *Salmonella* contamination, highlighting the public health risks associated with informal food vending systems [13]

In contrast, some studies have isolated organisms such as *Pseudomonas aeruginosa* from soymilk, reflecting variations in microbial profiles depending on environmental and processing conditions [16].

The co-occurrence of *B. cereus* and *S. typhi* underscores serious food safety concerns, as *B. cereus* can produce enterotoxins causing diarrheal illness, while *S. typhi* is a human pathogen responsible for typhoid fever [4, 5]. These findings are consistent with previous studies emphasizing the microbial hazards of street-vended foods [17, 18].

Notably, no fungal isolates were detected in any of the samples analyzed. The absence of fungi may result from shorter storage periods, limited environmental exposure, or competitive inhibition by bacterial populations, suggesting that bacterial contamination poses the primary health risk in these samples. This contrasts with previous reports identifying *Aspergillus* and *Candida* species in plant-based beverages and other street-vended foods, which are typically associated with prolonged storage or ambient environmental exposure [18].

Overall, this study underscores the urgent need for improved hygiene practices, proper processing techniques, and routine microbial monitoring in the production and vending of street-vended soymilk. Training vendors on food safety and raising consumer awareness of potential risks are critical strategies to mitigate public health hazards associated with contaminated plant-based beverages.

Table 1 Phenotypic Identification of Bacterial Isolates

Colony Morphology							**Biochemical Test**								
Isolates ID	**SSA**	**MRS**	**MAC**	**MSA**	**NA**	**PDA**	**Gram reaction**	**Oxidase**	**Catalase**	**Indole**	**Methyl red**	**VP**	**citrate**	**urease**	**Preliminary ID**
SMI	-	Creamy color colonies	-	Pink colonies	Cream colonies	-	+ rod shape	-	+	-	-	+	+	-	*Bacillus* sp.
SMIII	Colorless With black center	-	Colorless Colonies	-	Medium sized off white colonies	-	- rod shape	-	+	-	+	-	-	-	*Salmonella* sp.

Key: SSA= Salmonella Shigella Agar, MRS= De Man, Rogosa, and Sharpe Agar, MSA=Mannitol Salt Agar, NA=Nutrient Agar, PDA=Potato Dextrose Agar, VP=Voges- Proskauer

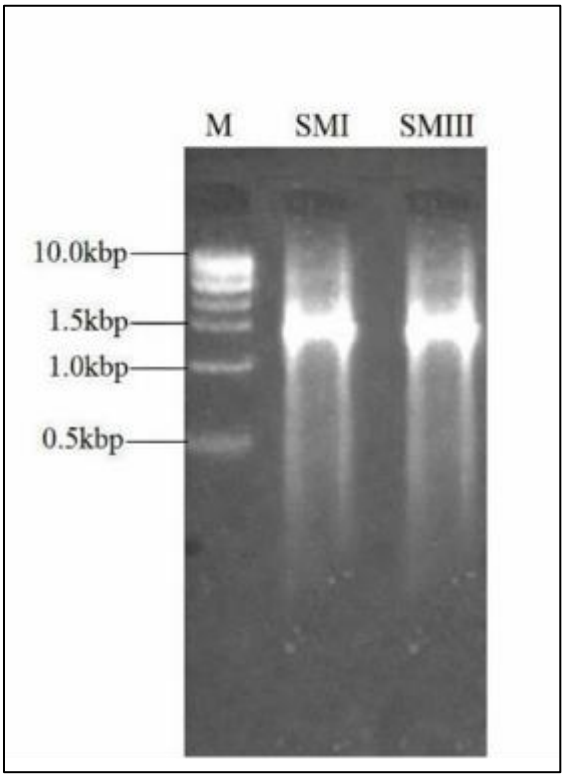
Table 2 Bacterial Load in Soymilk Samples

Sample ID	**Total Bacterial Count (CFU/mL)**	**Preliminary ID**
SMI	3.2×10^6	*Bacillus* sp.
SMIII	4.8×10^7	*Salmonella* sp.

Note: All samples were negative for fungal contamination

Table 3 Molecular Identification of Bacterial Isolates

Isolate ID	Genus	Species	Reference Strain	Sequence Similarity (%)
SMI	*Bacillus*	*Bacillus cereus*	12GK	90.27
SMIII	*Salmonella*	*Salmonella typhi*	NCTC 5760	98.4



Key: The lane M is a 1kbp DNA ladder; SMI= *Bacillus cereus* SMI; SMIII= *Salmonella typhi* SMIII

Figure 1 Gel image showing the amplification of 16SrRNA gene at 1.5kbp by the Bacterial Isolates

3.2. Antibiotic Susceptibility Testing

The antibiotic susceptibility profile of the bacterial isolates recovered from street-vended soymilk revealed important insights into their clinical relevance and potential public health implications. The *Salmonella typhi* SMIII demonstrated high susceptibility to ciprofloxacin (28 mm), ceftriaxone (25 mm), and chloramphenicol (22 mm), indicating that these antibiotics remain effective therapeutic options against the organism. This finding aligns with previous studies that reported fluoroquinolones and third-generation cephalosporins as effective treatments for *Salmonella* infections [4]. The observed susceptibility suggests that these antibiotics can still be relied upon for the management of typhoid fever in similar settings.

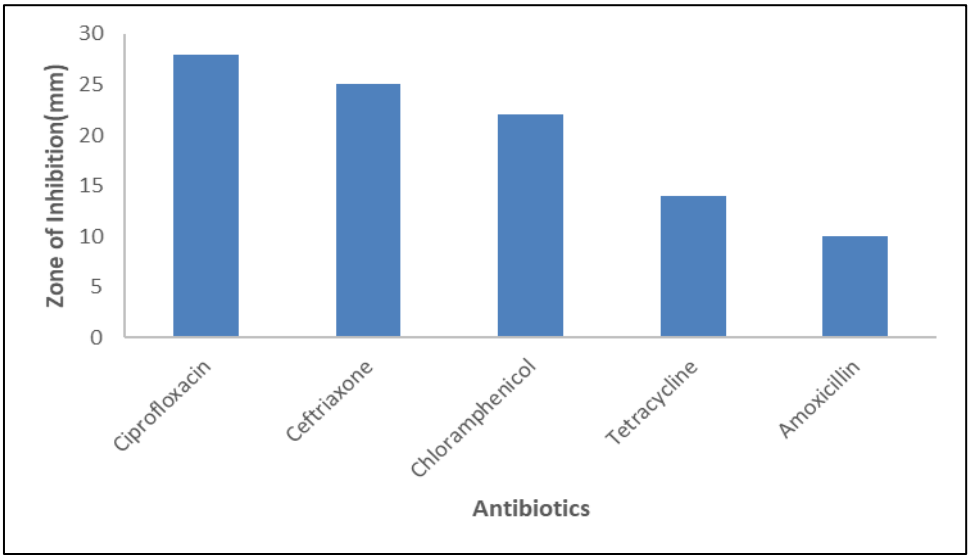
However, *S. typhi* SMIII exhibited resistance to amoxicillin (10 mm), which may be attributed to the production of β-lactamase enzymes that inactivate β-lactam antibiotics [7].

This resistance pattern is consistent with reports of increasing resistance of *Salmonella* species to commonly used antibiotics in developing countries, largely due to the misuse and overuse of antimicrobial agents [19]. The intermediate response to tetracycline (14 mm) suggests reduced susceptibility, indicating that tetracycline may not be a reliable first-line treatment option for infections caused by this isolate.

Similarly, the *Bacillus cereus* SMI isolate showed strong susceptibility to gentamicin (26 mm), vancomycin (24 mm), and erythromycin (20 mm). These findings are in agreement with earlier studies which reported that *B. cereus* is generally sensitive to aminoglycosides, glycopeptides, and macrolides [5]. The effectiveness of these antibiotics suggests their potential usefulness in treating infections caused by this organism.

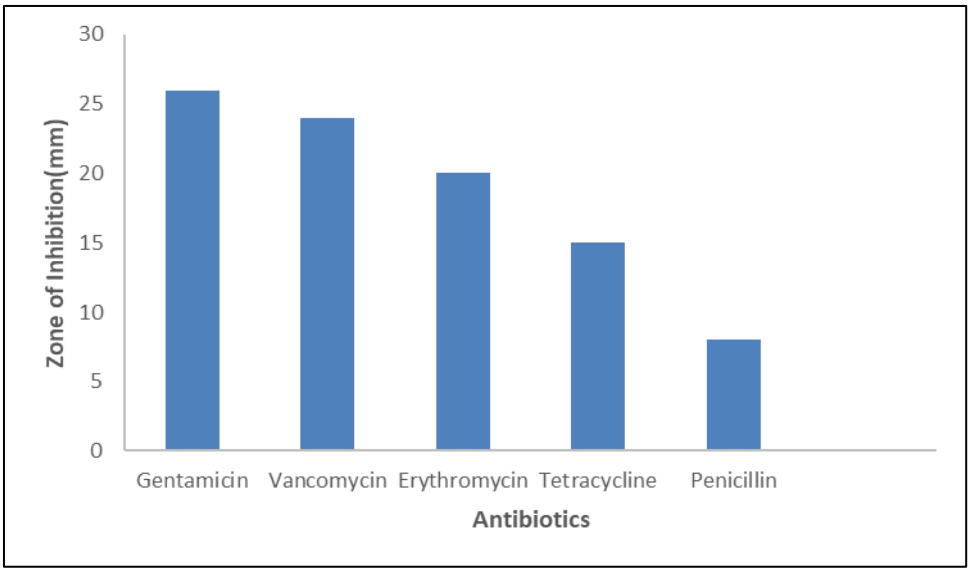
Conversely, *B. cereus* SMI demonstrated resistance to penicillin (8 mm), which is a well-documented characteristic of this species due to its ability to produce β-lactamases [20]. The intermediate susceptibility to tetracycline (15 mm) further indicates a possible emerging resistance trend, which could limit treatment options if not properly monitored.

Overall, the presence of antibiotic-resistant and intermediate strains among the isolates highlights a growing public health concern. The contamination of street-vended soymilk with such organisms not only increases the risk of foodborne infections but also contributes to the dissemination of antimicrobial resistance. These findings underscore the need for continuous surveillance, strict hygienic practices in food handling, and rational use of antibiotics to mitigate the spread of resistant pathogens [21].



Key: Zone ≥20 mm =Susceptible, Zone 15–19 mm = Intermediate, Zone ≤14 mm = Resistant; Ciprofloxacin, Ceftriaxone, Chloramphenicol=Susceptible; Tetracycline=Intermediate; Amoxicillin=Resistant

Figure 2 Antibiotic Susceptibility Profile of *Salmonella typhi* SMIII



Key: Zone ≥20 mm =Susceptible, Zone 15–19 mm = Intermediate, Zone ≤14 mm = Resistant; Gentamicin, Vancomycin, Erythromycin=Susceptible; Tetracycline=Intermediate; Penicillin =Resistant

Figure 3 Antibiotic Susceptibility Profile of *Bacillus cereus* SMI

4. Conclusion

Street-vended soymilk in the university community harbored pathogenic bacteria, which exhibited antibiotic resistance. This highlights the need for strict hygienic practices during production and handling, routine microbiological monitoring, and public health interventions to reduce contamination and protect consumers.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest exist among the authors

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