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Isolation and Characterization of *Bacillus* Species on Selected Cooked Foods Vended at Tarauni Market, Tarauni Local Government Area, Kano, Nigeria

Bako Pauline A[1]., Adamu Abdullahi S[1], Sadisu Farouk U[1] Aliyu Sani A[2]*

[1]*Department of Microbiology, Aliko Dangote University of Science and Technology, Wudil*
[2]*Department of Science Laboratory Technology, Aliko Dangote University of Science and Technology, Wudil*
**Corresponding Author Email/Phone: aliyujanzaki@gmail.com*

Abstract-

Foodborne diseases remain a significant public health concern, particularly in low- and middle-income countries where food safety practices are often inadequate. This study aimed to isolate and characterize *Bacillus* species in commonly consumed cooked foods vended at Tarauni Market, Kano State, Nigeria, and to evaluate their microbiological safety. A total of 25 food samples, including fried rice, jollof beans, Tuwon shinkafa, Tuwon masara, and spaghetti, were randomly collected from food vendors and subjected to bacteriological analysis. Viable mesophilic bacterial counts ranged from 1.03×10^4 to 7.09×10^4 CFU/g, while *Bacillus* counts ranged from 0.91×10^2 to 1.76×10^2 CFU/g. Molecular and biochemical analyses confirmed the presence of *Bacillus cereus* (68%), *B. mycoides* (24%), and *B. thuringiensis* (8%). The results indicate a high level of microbial contamination that exceeds acceptable limits for ready-to-eat foods. The detection of heat-resistant and potentially toxigenic *Bacillus* species underscores the risk of foodborne illness from street-vended foods. These findings highlight the urgent need for improved hygienic practices, vendor education, and regular microbiological surveillance to ensure food safety in open markets.

Keywords: *Bacillus cereus*; Cooked food; Microbial contamination; Tarauni Market; Food safety; Ready-to-eat foods.

1.0 Introduction

Foodborne illnesses are a growing global public health concern, often resulting from microbial contamination due to improper food handling, preparation, or storage. Among the causative agents, *Bacillus* species, particularly *Bacillus cereus*, are significant due to their ubiquity, spore-forming ability, and toxin production. In the United States alone, *Bacillus* species are estimated to cause over 60,000 cases of illness annually, primarily linked to the consumption of contaminated ready-to-eat or improperly stored foods [1].

B. cereus is a motile, Gram-positive, spore-forming bacterium commonly found in soil, water, and plant surfaces. Its spores can survive cooking temperatures and germinate during improper storage, particularly at temperatures above 30°C. Notably, the emetic toxin produced by some strains is heat-stable and may persist even after reheating [2,3]. Psychrotolerant strains of *B. cereus*, including *Bacillus weihenstephanensis*, pose an additional threat due to their ability to grow at refrigeration temperatures and produce toxins in cold-stored foods [4].

Epidemiological reports have linked *Bacillus* species to both diarrheal and emetic syndromes. The diarrheal form typically manifests 8–16 hours after ingestion and is characterized by abdominal pain and watery diarrhea, while the emetic form causes nausea and vomiting within 1–5 hours [5]. Foods frequently implicated include rice, pasta, beans, and meat dishes, particularly when stored under unsanitary conditions. Several studies have reported the persistence of *B. cereus* spores on food-contact surfaces and packaging materials, emphasizing the difficulty of complete elimination [6,7].

Despite the public health implications, routine testing for *B. cereus* remains limited in many developing regions. Moreover, food vendors often lack adequate training and access to potable water, increasing the risk of microbial contamination in cooked food sold in open markets [8].

In Nigeria, there is limited surveillance data on the presence of *Bacillus* species in vended foods, especially in highly populated urban markets such as Tarauni Market in Kano State. This study, therefore, aims to isolate and characterize *Bacillus* species present in selected cooked foods vended in Tarauni Market. The goal is to assess their prevalence, identify potential health risks, and provide data to inform food safety strategies and hygienic practices in local food vending environments.

2.0 Materials and Methods

2.1 Study Area

The study was conducted in Tarauni Local Government Area of Kano State, Nigeria. The area is located at approximately 8°32′38.44″E longitude and 11°57′31.03″N latitude, with

its administrative headquarters in Unguwa Uku within the Kano metropolis. Tarauni LGA covers an area of approximately 28 km² and had a population of 221,367 according to the 2006 census.

2.2 Sample Size

A total of 25 cooked food samples were randomly selected for analysis. These included fried rice, jollof beans, tuwon shinkafa (rice paste), tuwon masara (maize paste), and spaghetti, representing five commonly consumed dishes in the study area.

2.3 Sample Collection

Approximately 50 grams of each food sample were aseptically collected from 25 different food vendors within Tarauni Market using sterile containers. Vendors were selected using a simple random sampling method, ensuring a representative mix of food handlers. All samples were labeled and immediately transported in cold conditions to the Microbiology Laboratory at Bayero University, Kano, for analysis.

2.4 Sample Preparation and Serial Dilution

Each 25 g food sample was homogenized in 225 ml of sterile buffered peptone water. One milliliter (1 ml) of the homogenate was then serially diluted in 9 ml of sterile peptone water, producing dilution levels ranging from 10^{-1} to 10^{-5}. From each dilution, 1 ml was transferred in duplicates into sterile Petri dishes, which were appropriately labeled for subsequent analysis [15].

2.5 Viable Cell Count

Aerobic plate counts were performed using the pour plate method. One milliliter of each diluted sample was transferred into sterile Petri dishes, followed by the addition of molten Nutrient Agar. The contents were gently swirled and allowed to solidify. Plates were incubated at 37°C for 24 hours, after which colony-forming units (CFU) were counted and recorded [13].

2.6 Enumeration of *Bacillus* Species

Enumeration of *Bacillus* species was conducted using Mannitol Egg Yolk Polymyxin Agar (MYPA), prepared according to the manufacturer's instructions. A total of 21.5 g of MYPA powder was dissolved in 450 ml of distilled water and autoclaved at 121°C for 15 minutes. Once cooled to 45°C, 50 ml of sterile egg yolk emulsion and polymyxin B supplement were added. The medium was poured into sterile Petri dishes and inoculated with 1 ml of diluted sample. After solidification, plates were incubated at 37°C for 24 hours.

Colonies that appeared pinkish were counted, and the CFU/g was calculated based on the dilution factor [10].

2.7 Isolation and Identification of *Bacillus* Species

Following incubation, colonies with morphological characteristics suggestive of *Bacillus* species (e.g., pink colonies and lecithinase-positive zones on MYPA) were selected for further identification. Isolates underwent Gram staining and biochemical tests including catalase test, motility test, hemolysis, starch hydrolysis, gelatin liquefaction, growth on nutrient agar, and spore staining [15].

2.8 Molecular Screening for 16S rRNA Gene

Genomic DNA was extracted from bacterial isolates and used as templates for PCR amplification of the 16S rRNA gene. Each 25 µl reaction mixture contained 5 µl DNA template, 12 µl of PCR master mix (0.05 U/µL Taq polymerase, reaction buffer, 4 mM $MgCl_2$, and 0.4 mM of each dNTP), 1 µl each of forward and reverse primers, and 6 µl nuclease-free water. The PCR conditions involved initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 94°C (1 min), annealing at 55°C (1 min), extension at 72°C (1 min), and a final extension at 72°C for 7 minutes. PCR products were resolved on 1.8% agarose gel in 0.5× TAE buffer stained with ethidium bromide. A 100 bp DNA ladder was used as a molecular weight marker. Gels were run at 100 V for 50 minutes and visualized under UV light using a Uvitec documentation system (Cambridge, UK).

2.9 Statistical Analysis

Data obtained from microbial counts were subjected to a one-way Analysis of Variance (ANOVA). Means were compared using Least Significant Difference (LSD) at a 95% confidence level.

3.0 Results and Discussion

3.1 Results

The result of the total aerobic mesophilic counts and *Bacillus* count from the vended food samples in Tarauni market, Tarauni local government area, Kano state Nigeria was presented in Table 1 and Figure 1. A total of 25 samples comprising 5 samples from each of fried rice, *Tuwon Shinkafa*, *Tuwon masara*, Jollof beans, and Jollof spaghetti were microbiologically analyzed following the standard protocol of pour plate methods for viable bacterial counts.

Table 1. Total Aerobic Mesophilic and *Bacillus* Counts from the Sampled Food Vended in Tarauni Market, Tarauni Local Government Area, Kano State, Nigeria.

S/N	Samples	Sample Codes	TAMC (CFU/g)	TBC (CFU/g)
1.	Fried Rice	FR001	3.23×10^4	1.02×10^2
2.		FR002	1.92×10^4	0.91×10^2
3.		FR003	2.67×10^4	1.05×10^2
4.		FR004	2.33×10^4	1.11×10^2
5.		FR005	2.08×10^4	0.98×10^2
6.	*Tuwon Shinkafa*	TS001	7.09×10^4	1.23×10^2
7.		TS002	6.78×10^4	1.08×10^2
8.		TS003	5.23×10^4	1.11×10^2
9.		TS004	4.78×10^4	1.01×10^2
10.		TS005	5.12×10^4	1.06×10^2
11.	*Tuwon Masara*	TM001	3.56×10^4	1.13×10^2
12.		TM002	7.04×10^4	1.22×10^2
13.		TM003	6.33×10^4	1.41×10^2
14.		TM004	5.34×10^4	1.28×10^2
15.		TM005	4.89×10^4	1.24×10^2
16.	Jollof Beans	JB001	7.08×10^4	1.21×10^2
17.		JB002	6.81×10^4	1.32×10^2
18.		JB003	6.84×10^4	1.76×10^2
19.		JB004	6.56×10^4	1.36×10^2
20.		JB005	7.06×10^4	1.46×10^2
21.	Jollof Spaghetti	JS001	1.93×10^4	0.93×10^2
22.		JS002	1.03×10^4	1.01×10^2
23.		JS003	2.89×10^4	1.07×10^2
24.		JS004	2.07×10^4	1.02×10^2
25.		JS005	2.23×10^4	0.91×10^2
26.	NAFDAC/WHO STANDARD	-	1.0×10^2	-

Key: S/N – Serial number, TAMC – Total aerobic mesophilic counts, TBC – Total *Bacillus* counts, and CFU/g – colony forming unit per gram, FR – Fried Rice, TS – *Tuwon Shinkafa*, TM – *Tuwon Masara*, JB – Jollof Beans, JS – Jollof Spaghetti, WHO – World Health Organization.

The highest total aerobic mesophilic count was on sample TS001 (*Tuwon Shinkafa*, serial number 1) with 7.09 × 10^4 CFU/g of *Tuwon Shinkafa* while the least count was on sample JS002 (Jollof spaghetti, serial number 2) with 1.03 × 10^4 CFU/g of Jollof spaghetti.

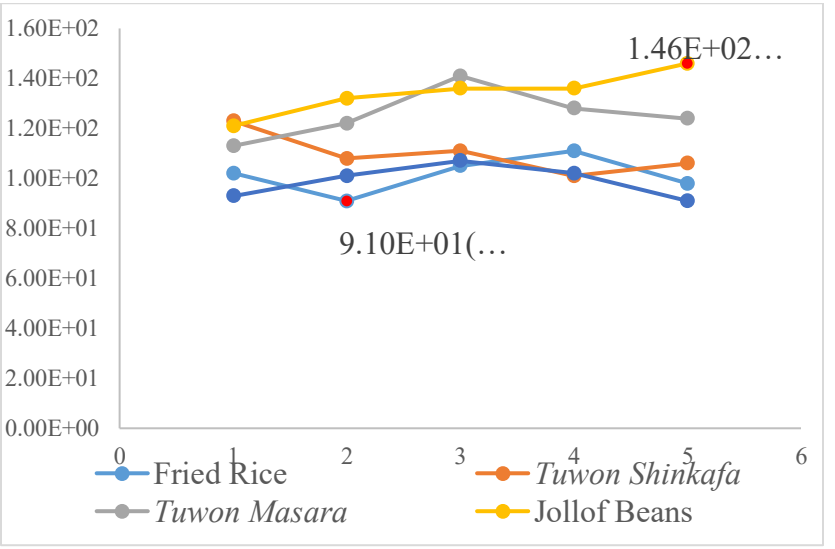

Figure 1. Total *Bacillus* Counts from the Sampled Food Vended in Tarauni Market, Tarauni Local Government Area, Kano State, Nigeria in (CFU/g).

However, the total *Bacillus* count was found to be higher on sample JB003 (Jollof beans, serial number 003) with 1.76 × 10^2 CFU/g of Jollof beans while the least count was on samples FR002 and JS005 (i.e. Fried rice serial number 002 and Jollof spaghetti serial number 005) with 0.91 × 10^2 CFU/g of the samples each.

The frequency of occurrence of three identified *Bacillus* species isolated from the vended food samples at Tarauni market in Tarauni local government area, Kano state Nigeria was presented in Table 2. In this study, *Bacillus cereus* was isolated from 17 samples of the analyzed vended food making a 68% of the total isolates, *Bacillus mycoides* was isolated in 6(24%) samples only, while 2(8%) of the sampled foods revealed the presence of *Bacillus thuringiensis* throughout.

Table 2. Frequency of occurrence of *Bacillus* species from the Sampled Food Vended in Tarauni Market, Tarauni Local Government Area, Kano State, Nigeria.

S/N	Samples	*Bacillus cereus*	*Bacillus mycoides*	*Bacillus thuringiensis*
1.	Fried Rice	3	2	0
2.	*Tuwon Shinkafa*	4	1	1
3.	*Tuwon Masara*	3	2	1
4.	Jollof Beans	5	2	0
5.	Jollof Spaghetti	2	0	0
6.	Total (%)	17(68)	6(24)	2(8)

In Figure 2, the frequency distribution of *Bacillus* species in the sampled food at Tarauni Market, Tarauni Local Government Area, and Kano State, Nigeria is illustrated based on the analyzed foods.

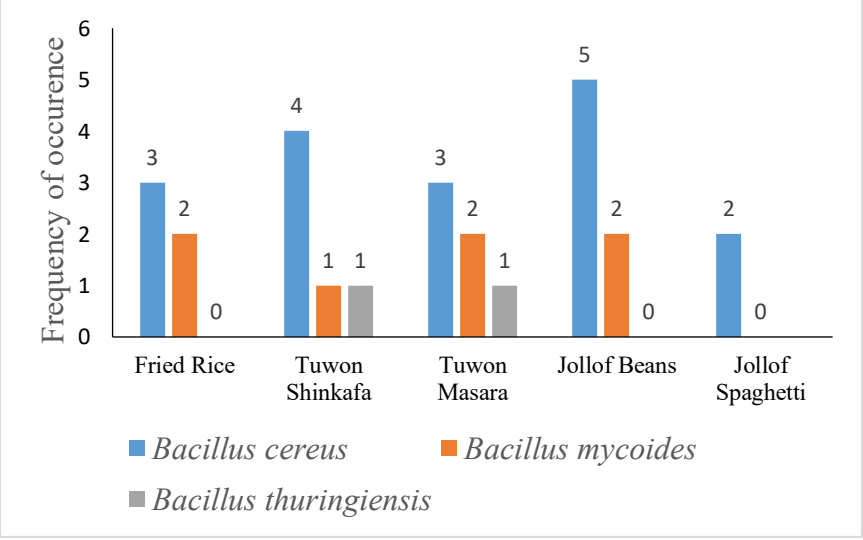

Figure 2. Frequency of occurrence of *Bacillus* species from the Sampled Food Vended in Tarauni Market, Tarauni Local Government Area, Kano State, Nigeria.

The presence of *Bacillus cereus* was notable across the samples, occurring in all 5 samples of Jollof beans, 4 samples of *Tuwon Shinkafa*, 3 samples each of fried rice and *Tuwon masara*, and only 2 samples of Jollof spaghetti. *Bacillus mycoides* was detected in 2 samples each of

fried rice, *Tuwon masara*, and Jollof beans, in 1 sample of *Tuwon Shinkafa*, and was absent in Jollof spaghetti. Additionally, *Bacillus thuringiensis* was identified once in samples of *Tuwon Shinkafa* and *Tuwon masara*, respectively.

Table 3 details the phenotypic and genotypic characteristics of different *Bacillus* species isolated from the food samples obtained at Tarauni Market in Kano State, Nigeria. Each row corresponds to a specific *Bacillus* isolate, detailing its characteristics based on various tests and observations conducted in the laboratory. The Table provides a comprehensive overview of the traits exhibited by different *Bacillus* species found in the sampled food from Tarauni Market, aiding in their identification and understanding of their potential characteristics.

Table 3. Phenotypic and Genotypic Characteristics of *Bacillus* Species Isolated from the Sampled of Food Vended at Tarauni Market, Tarauni Local Government Area, Kano State, Nigeria

S/N	Isolate Colonial morphology	Microscopic examination	GR	Mot	Cat	Hem	GLT	SHT	SST	ETV	EPH	16s rRNA similarity	Isolates
1.	Pink/purple colonies on MYPA plate	Rod shape	+	+	+	+	+	+	+	+	+	96%	*Bacillus cereu*
2.	Pinkish orange hairy rhizoid on MYPA plate	Rod shape	+	-	+	+	+	+	+	+	+	83%	*Bacillus mycoides*
3.	Whitish/grey slimy colonies on MYPA plate	Rod shape	+	+	+	+	+	+	+	+	+	88%	*Bacillus thuringiensis*

Key: GR – Gram's reaction; **Mot** – Motility test; **Cat** – Catalase test; **Hem** – Haemolysis test; **GLT** – Gelatin liquefaction test; **SHT** – Starch hydrolysis test; **SST** – Spore staining test; **ETV** – Effect of temperature on vegetative growth; **EPH** – Effect of pH on growth; **rRNA** – Ribosomal ribonucleic acid.

The PCR amplification of the 16S rRNA gene yielded distinct bands, confirming the presence of *Bacillus* species. The gel electrophoresis image is shown in Plate 1.

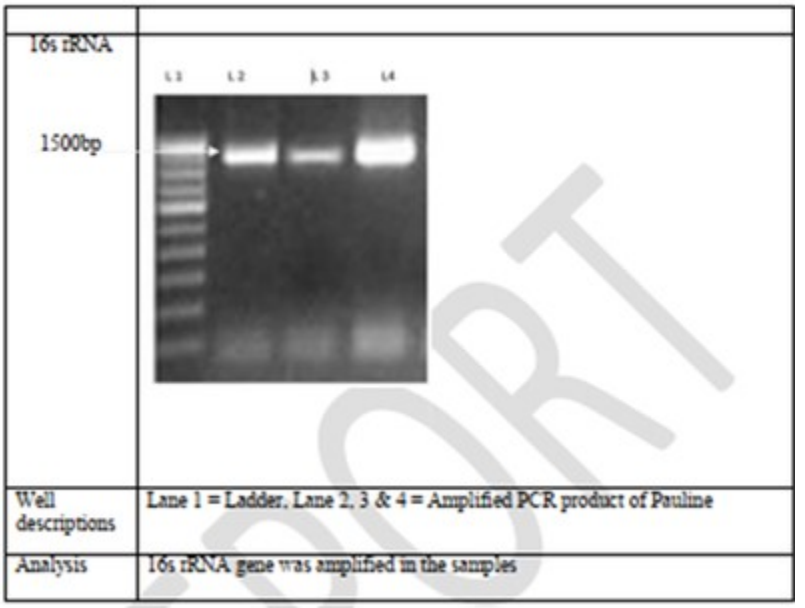

Plate 1. Gel Electrophoresis of the Amplified 16s rRNA gene

3.2 Discussion

The findings from this study reveal that foods vended at Tarauni Market in Kano State are subject to significant microbial contamination, likely due to unsanitary handling and environmental exposure. Many vendors operate in open-air settings with poor hygiene infrastructure, leading to potential contamination from dust, insects, and human contact. All food samples analyzed showed total aerobic mesophilic counts ranging from 1.03×10^4 to 7.09×10^4 CFU/g, exceeding the <10 CFU/g standard recommended for ready-to-eat foods. These values are consistent with similar studies in Tamale, Ghana [16], and other parts of Nigeria including Maiduguri [17] and Wudil Campus, KUST [18].

The elevated bacterial loads suggest inadequate food handling practices and underscore the importance of implementing stricter hygiene standards in such market environments. Contributing factors may include type of food, holding time before sale, temperature, and personal hygiene of vendors, as previously reported by Mahfuza et al. [19].

More importantly, the study confirms the presence of foodborne *Bacillus* species, particularly *B. cereus*, *B. mycoides*, and *B. thuringiensis*, in all sampled food types. These organisms are known to cause two types of foodborne illness: diarrheal and emetic syndromes [20, 21]. The diarrheal form resembles *Clostridium perfringens* poisoning, while the emetic form is similar to *Staphylococcus aureus* intoxication and can be fatal in severe cases [22]. The presence of these species in fried rice, beans, Tuwon Shinkafa, Tuwon Masara, and spaghetti indicates widespread contamination and a potential public health risk.

Bacillus cereus was the most frequently isolated species, found in 68% of samples, with CFU values ranging from 0.91×10^2 to 1.76×10^2 CFU/g. This level approaches or exceeds the threshold of 10^3 CFU/g often cited as unsafe for consumption [23]. These findings are higher than those reported by Kaur et al. [24] (26.89% contamination) and Xiong et al. [25] (6.34% contamination), suggesting a more serious contamination profile in the study area.

The pathogenicity of *Bacillus* species is complex and influenced by strain variability in morphology, metabolism, and environmental tolerance [26, 27]. In this study, 25 isolates were characterized phenotypically and biochemically. Observed differences in haemolysis, starch hydrolysis, and gelatin liquefaction suggest metabolic diversity among the isolates. Notably, all ten classical strains identified were non-mannitol fermenting and β-haemolytic, consistent with virulent *Bacillus* profiles. The ability (or inability) to hydrolyze starch and liquefy gelatin has been linked to the presence of emetic toxin-producing strains [28].

Environmental tolerance testing revealed that all isolates grew optimally at 28°C and 37°C, but none grew at 4°C. Growth was supported between pH 6 and 8, with limited or no growth at pH 4 or 9. These findings align with previous studies by Khater et al. [26], Martinović et al. [27], and Cunha et al. [23], confirming that *Bacillus* strains are well adapted to ambient food storage conditions, making cold chain maintenance critical.

Heat resistance testing showed that all isolates survived up to 60°C, with variable resistance at higher temperatures, indicating that improper cooking or reheating may fail to inactivate spores. The literature corroborates that *Bacillus* spores, especially those of *B. cereus*, exhibit a broad range of heat resistance [29]. This reinforces the need for rigorous temperature control during food preparation and storage.

The health risk posed by these strains is amplified by the lack of routine food monitoring and poor hygiene infrastructure in the study area. Observational findings further reveal significant environmental challenges at Tarauni Market, including overcrowding, inconsistent water supply, and reliance on potentially contaminated water sources. Vendors often store water in open containers, facilitating microbial transmission. This situational analysis supports the need for targeted interventions, including regular health inspections, education for food vendors, and improved access to clean water.

In summary, the presence of pathogenic and heat-resistant *Bacillus* strains in ready-to-eat foods, coupled with substandard hygiene practices, presents a public health risk in Tarauni Market. Strengthening food safety awareness and enforcing sanitation regulations are critical steps toward reducing foodborne disease incidence in this community.

4.0 Conclusion and Recommendations

4.1 Conclusion

This study has demonstrated a high prevalence of microbial contamination in cooked foods vended at Tarauni Market, Kano State. All analyzed samples were found to contain *Bacillus* species, notably *B. cereus*, *B. mycoides*, and *B. thuringiensis*. The total aerobic mesophilic counts ranged from 1.03×10^4 to 7.09×10^4 CFU/g, while *Bacillus* counts ranged from 0.91×10^2 to 1.76×10^2 CFU/g—both exceeding recommended safety limits for ready-to-eat foods.

The presence of heat-tolerant and metabolically diverse *Bacillus* strains highlights the potential for foodborne illness if proper preventive measures are not implemented. Environmental observations suggest that factors such as inadequate hygiene, inconsistent water supply, and poor food storage practices contribute to this public health risk.

4.2 Recommendations

In view of the findings, the following recommendations are proposed to improve food safety and reduce the risk of *Bacillus*-related foodborne illnesses in local markets:

i. **Routine Microbiological Surveillance:** Regular monitoring and microbial assessment of vended foods should be implemented by local health authorities.

ii. **Food Handler Education:** Structured training programs should be provided for food vendors on hygienic practices, safe food preparation, and temperature control.

iii. **Improved Sanitation Infrastructure:** The provision of clean water sources, hand-washing stations, and proper waste disposal systems in market environments is essential.

iv. **Policy Enforcement:** Regulatory bodies should conduct regular inspections and enforce compliance with food safety standards among vendors and handlers.

v. **Further Research:** Future studies should investigate the toxin gene profiles and antibiotic resistance of *Bacillus* isolates to better understand their public health implications.

Taking these measures makes it possible to significantly enhance the microbial quality of street-vended foods and protect public health in densely populated urban settings like Tarauni.

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