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Original Research Article

Isolation and Identification of Some Pathogenic Bacteria from *Cassava* (*Manihot Esculenta*) Product (*Fufu*) In Local Eateries in Amassoma Community

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Abstract
Cassava-based foods such as cassava (Manihot esculenta) fufu are a major dietary component in Nigeria, particularly in Amassoma, Bayelsa State. Despite undergoing fermentation a process that supports the growth of beneficial lactic acid bacteria and detoxifies harmful compounds, cassava (Manihot esculenta) fufu remains susceptible to microbial contamination during post-processing. This study aims to isolate and identify bacteria species present in cassava (Manihot esculenta) fufu sold in local eateries in Amassoma to assess its microbial quality and associated health risks. Samples were collected from three vendors, analyzed for bacterial load, and subjected to biochemical identification procedures. Results revealed the presence of potentially pathogenic bacteria, including Escherichia coli, Klebsiella spp., Staphylococcus spp., and Bacillus spp., with E. coli and Bacillus spp showing the highest prevalence (30% each). Proximate analysis indicated cassava (Manihot esculenta) fufu high carbohydrate content (84.99%) with low protein and fiber. The findings underscore the urgent need for improved hygiene practices, clean water usage, and routine microbial assessments in informal food vending to ensure consumer safety.

Keywords: *Cassava-based foods, local eateries, Manihot esculenta, pathogenic bacteria.*

INTRODUCTION
Cassava (*Manihot esculenta*) is a widely grown crop in sub-Saharan Africa, especially in Nigeria, where it serves as a major food source for millions of people. It is processed into different food products such as *cassava* (*Manihot esculenta*) *fufu*, *garri*, and *tapioca*. Among these, *cassava* (*Manihot esculenta*) *fufu* is popular because of its smooth texture and ability to complement various soups. The preparation of *cassava* (*Manihot esculenta*) *fufu* involves fermentation, an essential process that improves its texture, reduces harmful cyanogenic compounds, and enhances its flavor (Obilie et al., 2004).

Fermentation is mainly driven by lactic acid bacteria (LAB) such as *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, and *Pediococcus* spp. These bacteria help lower the pH and prevent the growth of harmful microorganisms (Amoa-Awua et al., 1996). However, despite the fermentation process, *cassava* (*Manihot esculenta*) *fufu* can still become contaminated if proper hygiene is not maintained during processing, storage, or serving. Poor handling practices can introduce harmful bacteria such as *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*, which can cause foodborne illnesses (Adesetan et al.,2013).

In Amassoma, a university town in Bayelsa State, *cassava* (*Manihot esculenta*) *fufu* is a common meal served in local eateries, catering to both students and residents. However, many of these eateries operate informally, with little attention to food safety regulations, increasing the risk of bacterial contamination (Adepoju et al., 2018). Contaminated *cassava* (*Manihot esculenta*) *fufu* can serve as a medium for disease causing bacteria, especially in areas where food hygiene awareness is low (WHO, 2021).

Despite the widespread consumption of *cassava* (*Manihot esculenta*) *fufu*, there has been little research on its microbial safety in local eateries, particularly in Amassoma. Most studies focus on the fermentation process, ignoring potential contamination that may occur afterward (Omemu & Bankole, 2015). This study seeks to address this gap by identifying pathogenic bacteria present in *cassava* (*Manihot esculenta*) *fufu* sold in eateries in Amassoma and evaluating its microbial quality.

MATERIALS AND METHODS

Sample collection

Freshly cooked *cassava* (*Manihot esculenta*) *fufu* samples of about 50 grams each rapped in nylon were collected aseptically from three different local eateries in amassoma, bayelsa state, Nigeria. It was brought to the Microbiology laboratory, Department of Microbiology, Faculty of Science, University of Port Harcourt for analysis.

Total Heterotrophic Bacterial (THB) Count

A 10g sample of cassava (*Manihot esculenta Crantz*) fufu was mixed with 90 mL of sterile saltwater (0.85% NaCl) under clean conditions. Using a clean pipette, 0.1 mL of the diluted sample was placed onto Nutrient Agar (NA) plates and spread evenly with a sterile glass rod. The plates were kept at 37°C for 24 hours. After incubation, the visible bacterial colonies were counted to calculate the colony-forming units (CFU) per gram of starch. Different-looking colonies were picked and streaked onto new agar plates to grow pure cultures, which were incubated for another 24 hours. These pure cultures were then analyzed under a microscope using Gram staining and tested biochemically to identify the bacteria (Cheesbrough, 2006; Li et al*.,* 2022).

Gram staining and Biochemical tests

The procedure was done following *Prescott et al.,* 2008 and biochemical tests (catalase, citrate, indole, Methyl red and Voges-Proskauer, oxidase, Triple sugar iron agar and Sugar fermentation tests were also carried out (Moses *et al.,* 2023; Iwu *et al.,* 2021; Oladimeji *et al.,*2022; Okoro *et al.,* 2021).

Proximate Analysis of Samples

The following parameters (moisture, ash, crude protein, nitrogen, crude fiber, and carbohydrate content) were assayed (AOAC, 2019; Musa *et al.,* 2023).

Spore Staining Test

The spore test was employed to detect spore-forming bacteria, particularly those belonging to the genus Bacillus, which are significant due to their resistance to heat and desiccation. The Schaeffer-Fulton method was used, involving primary staining with 1% malachite green under steam heat for 5 minutes, followed by counterstaining with safranin. Under microscopic examination, endospores appeared green, while vegetative cells stained red (Moses et al., 2023).

RESULTS

Table 1: Total colony count of bacteria isolated from *cassava* (*Manihot esculenta*) *fufu*

SAMPLES	DILUTION	PLATE	PLATE 2	PLATE 3	TOTAL	AVERAGE	CFU/mL	log_{10}(CFU)
Vendor 1a	10^{-3}	113	79	19	211	70.3	7.0×10^5	5.85
Vendor 1b	10^{-3}	23	24	23	70	23.3	2.3×10^5	5.36
Vendor 1c	10^{-3}	27	26	27	80	26.7	2.7×10^5	5.43
Vendor 2a	10^{-3}	9	8	4	21	7	7×10^4	4.85
Vendor 2b	10^{-3}	1	17	3	21	7	7×10^4	4.85
Vendor 2c	10^{-3}	6	46	6	58	19.3	1.9×10^5	5.28
Vendor 3a	10^{-3}	3	3	9	15	3	3×10^4	4.48
Vendor 3b	10^{-3}	3	1	12	16	5.3	5×10^4	4.7
Vendor 3c	10^{-3}	7	11	5	23	7.7	7.7×105	5.89

Table 1 shows the total colony count per mL of microorganisms isolated from *cassava* (*Manihot esculenta*) *fufu* samples ranging from 3×10^4 to 7.0×10^5 CFU/mL. The highest count total count of 7.0×10^5 CFU/mL was observed in Vendor 1a, while the lowest was 3×10^4 CFU/mL (Vendor 3a).

Table 2: Gram reaction and biochemical characterization of bacteria isolates

S/N	IC	GR	CM	Cat	Ox	Ind	Spore	Cit	MR	VP	Gas	H_2S	Slant	Butt	Glu	Lac	Suc	Mal	PO
1	1Aa	-	Rod	+	-	-	-	-	+	-	+	-	A	A	A/G	A/G	A/G	A/G	*E.coli*
2	1Ab	-	Rod	+	-	-	-	+	-	+	+	-	A	A	A/G	A/G	A/G	A/G	*Klebsiella* spp
3	1Ba	-	Rod	+	-	-	-	-	+	-	+	-	A	A	A/G	A/G	A/G	A/G	*E.coli*
4	1Bb	+	Rod	+	-	-	+	+	+	+	-	-	K	A	A	A	-	-	*Bacillus* spp
5	1Bc	+	Cocci	+	-	-	-	+	-	+	-	-	A	A	A	A	A	A	*Staphylococcus* spp
6	1Ca	+	Rod	+	-	-	+	+	+	+	-	-	K	A	A	A	-	-	*Bacillus* spp
7	1Cb	-	Rod	+	-	-	-	-	+	-	+	-	A	A	A/G	A/G	A/G	A/G	*E.coli*
8	2Aa	+	Cocci	+	-	-	-	+	-	+	-	-	A	A	A	A	A	A	*Staphylococcus* spp
9	2Ab	+	Rod	+	-	-	+	+	+	+	-	-	K	A	A	A	-	-	*Bacillus* spp
10	2Ba	-	Rod	+	-	-	-	+	-	+	+	-	A	A	A/G	A/G	A/G	A/G	*Klebsiella* spp
11	2Bb	+	Rod	+	-	-	+	+	+	+	-	-	K	A	A	A	-	-	*Bacillus* spp
12	2Ca	-	Rod	+	-	-	-	-	+	-	+	-	A	A	A/G	A/G	A/G	A/G	*E.coli*
13	2Cb	+	Cocci	+	-	-	-	+	-	+	-	-	A	A	A	A	A	A	*Staphylococcus* spp
14	3Aa	-	Rod	+	-	+	-	-	+	-	+	-	A	A	A/G	A/G	A/G	A/G	*E.coli*
15	3Ab	+	Rod	+	-	-	+	+	+	+	-	-	K	A	A	A	-	-	*Bacillus* spp
16	3Ba	-	Rod	+	-	-	-	+	-	+	+	-	A	A	A/G	A/G	A/G	A/G	*Klebsiella* spp
17	3Bb	+	Cocci	+	-	-	-	+	-	+	-	-	A	A	A	A	A	A	*Staphylococcus* spp
18	3Ca	+	Rod	+	-	-	+	+	+	+	-	-	K	A	A	A	-	-	*Bacillus* spp
19	2Bb	-	Rod	+	-	-	-	-	+	-	+	-	A	A	A/G	A/G	A/G	A/G	*E.coli*
20	2Bc	+	Cocci	+	-	-	-	+	-	+	-	-	A	A	A	A	A	A	*Staphylococcus* spp

KEY: IC; Isolate code**, GR;** Gram reaction**, CM;** Cell morphology**, Cat;** Catalase**, Ox;** Oxidase**, Ind;** Indole**, Cit;** Citrate**, MR;** Methyl red**, VP;** Vogesproskauer**, H_2S;** Hydrogen sulphide**, Glu;** Glucose**, Lac;** Lactose**, Suc;** Sucrose**, Mal;** maltose**, PO;** Probable Organism; **-**, negative; **+**, positive, **A**= acid, **B**= base, **A/G**= acid and gas.

Table 2 summarizes the biochemical and morphological characteristics used for the identification of bacterial isolates from various *cassava* (*Manihot esculenta*) fufu samples, designated as "1Aa" through "2Bc". Each isolate was tested for several traits including Gram reaction (GR), cell morphology (CM), catalase (Cat), oxidase (Ox), indole production (Ind), spore formation (Spore), citrate utilization (Cit), methyl red (MR), Voges-Proskauer (VP), gas and hydrogen sulfide (H_2S) production, Triple Sugar Iron (TSI) slant/butt reactions, and sugar fermentation abilities (glucose, lactose, sucrose, maltose), among others**.**

Table 3: Proximate Analysis of *cassava* (*Manihot esculenta*) fufu

PARAMETERS	*Cassava (Manihot esculenta) fufu*
Moisture (%)	8.3
Ash content (%)	1.97
Fat & Oil (%)	1.3
Protein (%)	1.83
Carbohydrate (%)	84.99
Crude Fiber (%)	1.61

Table 3 presents the proximate analysis of *cassava* (*Manihot esculenta*) *fufu*) and the analysis reveals that *cassava* (*Manihot esculenta*) *fufu* is predominantly composed of carbohydrates, accounting for a significant 84.99% of its content. Moisture is the second highest component, measured at 8.3%. The remaining components are present in relatively small percentages: protein at 1.83%, ash content at 1.97%, crude fiber at 1.61%, and fat & oil at 1.30%.

Discussion

The microbiological analysis of *cassava* (*Manihot esculenta*) *fufu* samples from eateries in Amassoma revealed varying levels of bacteria contamination. The presence of *Escherichia coli*, *Klebsiella* spp, *Staphylococcus* spp and *Bacillus* spp aligns with previous studies on the microbial ecology of fermented cassava products. The detection of these organisms raises concerns about food safety and hygiene practices in local food vending (Achi & Ukwuru, 2015; Olaniran et al., 2017).

The isolation of *E. coli* (30%) and *Klebsiella* spp (15%) in this study is consistent with findings from Adebayo-Tayo et al*.*, (2012), who reported enteric bacteria in *cassava* (*Manihot esculenta*) *fufu* samples due to contamination from water, handling, and processing equipment. *E*. coli is a major indicator of fecal contamination, suggesting improper handling and potential risks of foodborne diseases (Adeleke et al*.*, 2020). Similarly, *Klebsiella* spp has been identified in fermented foods and is known to cause opportunistic infections, particularly in immunocompromised individuals (Olaniran et al*.*, 2017).

The presence of *Staphylococcus* spp (25%) in the samples is indicative of possible post-fermentation contamination. This bacterium is commonly found on human skin and can be introduced into food through direct contact during processing or serving (Nwachukwu et al*.*, 2010). Some strains of *Staphylococcus aureus* produce enterotoxins that can cause food poisoning, characterized by nausea, vomiting, and diarrhea (Achi & Ukwuru, 2015).

The detection of *Bacillus* spp (30%) suggests its role in the natural fermentation of cassava. *Bacillus* species are known to produce enzymes that break down cassava starch into fermentable sugars, contributing to the unique taste and texture of *cassava* (*Manihot esculenta*) *fufu*. However, certain strains such as *Bacillus cereus*, can produce toxins that cause foodborne illnesses. The presence of *Bacillus* spp in this study highlights the need for proper monitoring of fermentation conditions to minimize potential risks.

The identification of *E. coli* and *Klebsiella* spp in *cassava* (*Manihot esculenta*) *fufu* samples suggests potential risks of gastrointestinal infections, particularly when these foods are consumed without proper heating. Pathogenic strains of *E. coli* can cause diarrhea, urinary tract infections, and in severe cases, hemolytic uremic syndrome (HUS) (Adeleke et al*.*, 2020). Similarly, *Klebsiella* spp has been implicated in pneumonia, wound infections, and bloodstream infections, especially in hospital environments (Olaniran et al*.*, 2017).

Staphylococcus spp contamination indicates poor hygiene practices among food handlers. Studies have shown that *Staphylococcus aureus* can survive in cooked foods, and its enterotoxins remain stable even at high temperatures (Nwachukwu et al*.*, 2010). This highlights the importance of maintaining strict hygiene measures in food preparation and vending.

The variation in bacterial counts among vendors suggests differences in hygiene practices and environmental conditions. Vendors with high bacterial loads, such as Vendor 1a (7.0×10^5 CFU), may have inadequate sanitation measures, contaminated water sources, or prolonged exposure of *cassava* (*Manihot esculenta*) *fufu* to open air. In contrast, vendors with lower bacterial counts, such as Vendor 3a (3×10^4 CFU), may adhere to better hygiene protocols.

Previous studies have identified several factors that contribute to microbial contamination in cassava products, including use of contaminated water during fermentation and processing (Adebayo-Tayo et al*.*, 2012); Poor personal hygiene among food handlers (Nwachukwu et al*.*, 2010) and exposure of *cassava* (*Manihot esculenta*) *fufu* to environmental contaminants during storage and display (Achi & Ukwuru, 2015).

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