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Microbial Quality Assessment of Locally Produced Millet Drink "*Lamugin*" and *Hibiscus sabdariffa* Calyxes Extract Beverage "*Sobolo*" in Accra, Ghana

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Abstract

Local Ghanaian beverages such as *Sobolo* and *Lamugin*, play a significant cultural, economic, and medicinal role in the daily lives of the population. These traditional drinks are produced using locally sourced ingredients, making them both affordable and accessible. The COVID-19 pandemic further heightened the economic importance of these beverages, as demand surged due to their perceived immune-boosting properties. However, the production of these beverages often occurs in non-sterile environments with minimal regulatory oversight, leading to concerns about microbial contamination. This study aimed to evaluate the microbial quality of *Sobolo* and *Lamugin* sold at selected street markets in the Greater Accra region of Ghana. Samples were collected from various vendors and analyzed for bacterial and fungal pathogens using standard microbiological techniques. The results revealed significant microbial contamination in many samples, with bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* detected at levels exceeding safe limits. These findings raise serious public health concerns, as the consumption of contaminated beverages could lead to gastrointestinal infections and other health issues. This highlights the urgent need for stricter regulations and improved hygiene practices in the production of local beverages to ensure their safety for consumers. Implementing better regulatory frameworks, regular

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inspections, and public awareness campaigns could help mitigate the risks associated with these popular drinks, ensuring they remain a safe and cherished part of Ghana's cultural heritage.

Keywords

Lamugin, Sobolo, Bacteria Contamination

1. Introduction

Local drinks serve as refreshments around the world including Ghana. They are historically inspired drinks made using ingredients sourced locally and prepared using time-tested techniques that regularly offer the great majority of the population a range of incredibly affordable and easily available drinks. Albeit there are no standardized protocols for production to ensure safety, these locally produced beverages play a significant role in satisfying the demands of urban residents and quenching their thirst, especially among the younger generation [1] [2].

The most popular locally produced drinks or beverages in Ghana, are non-alcoholics including *Lamogine* (produced from milled corn with ginger and sugar), *Sobolo* (from the dried petals of the hibiscus flower), *atadwe* milk (from tiger nuts), *Emuduro* or Ginger beer and *Brukina* (fermented millet and milk smoothie) and *Abele* walls (a local popsicle from frozen milkshake produced from powdered milk with added sugar), *Asana* (non-alcoholic beverage made from extract of fermented crushed corn which has been boiled with caramelized sugar) and the alcoholic *Burukutu* (from sorghum grains) and *Pito* (produced from guinea corn and/or millet grains) [2] [3]. These beverages are mostly sold commercially on the streets and in stores by hawkers and merchants in the vicinity of public spaces like marketplaces [2] [4] and occasionally served at special events like weddings, parties, and funerals because of their affordability.

Because some of the ingredients for the production of these beverages are claimed to have medicinal properties [5]-[7], some people in Ghana utilize them in place of traditional medication to treat a range of physical conditions, such as cancers, headaches, abscesses, billows, and insomnia. *Sobolo*, for instance, has been shown to possess antimicrobial properties [7]. Moreover, *Emuduro* has been shown to have antimicrobial, antioxidant, and anti-neuroinflammatory properties [6]. It also alleviates pain, nausea, and vomiting, among other common health issues [5]. Most recently, *Sobolo* has been used as an immune booster against COVID-19 due to claims that the extract from Hibiscus petals boosts immunity. Nevertheless, a study exploring the effect of Hibiscus flower petals on hematocrit, platelets, and erythrocytes found no significant effect [8]. Nevertheless, *Sobolo* remains one of the most popular locally produced beverages in Ghana [4].

The *Hibiscus sabdariffa* whose dried petals are used to produce *Sobolo* belongs to the Malvaceae family of herbaceous plants primarily grown in tropical and

subtropical climates [9]. The petal extract is extremely rich with organic acids, phytosterols, and polyphenols, vitamins, minerals, and bioactive substances [7]. The beverage prepared from the petals of this plant has several names in different places, including the *Sobolo* in Ghana, *Sorrel* in Guinea, *Bissap* in Senegal, *Wonjo* in The Gambia and *Flora* in Jamaica. The preparation involves the extraction of the prominent reddish pigment and flavor through boiling and the addition of sugar or honey as a sweetener to taste and/or spices including ginger, moringa and taken either hot or cold for its numerous salubrious benefits. For commercial purposes, it is packaged in plastic bottles, refrigerated, and sold on the streets [1] [10] (see **Table 1**). The production process until bottling is mostly done in individual homes without following any restricted aseptic guidelines making it prone to contamination [3] [4] [9] [11].

Lamugin is another traditional beverage found in Ghana produced from millet by simply blending the millet with desired spices including ginger, pepper, and cloves and straining the essence into clean bottles with the addition of sugar or honey for taste. The production process for *Lamugin* is relatively simpler compared to *Sobolo* but is similarly produced in homes of people with simple household tools without any external regulations to avoid potential [12].

Table 1. Street Beverages analyzed in the study.

Beverage	Description	Cooking method	Handling after cooking	Sources of contamination
Sobolo	Extracted by-products	Boiling	Served in a bottle	Cooking utensils, bottle, water, spices,
Lamugin	Fomented millet	Blending	In bottle	Equipment, spoon, hands, water

The economic benefit of these drinks in Ghana was heightened during the COVID-19 era due to the purported ability to improve immunity against the virus [13]. This led to a mad rush for these products and an economic boon for the producers and/or sellers. However, the upsurge and consumption of these beverages under uncontrolled conditions with potentially unhygienic packaging materials for distribution could lead to microbial contaminations with its accompanied potential public health implications without regulations [14] [15]. Given the appreciably high patronage for *Sobolo* and *Lamugin* during the covid-19 era, there was need to determine its microbial safety in the country. We thence sought to evaluate the microbial quality of *Sobolo* and *Lamugin* sold at selected street markets in the Greater Accra region of Ghana.

2. Methodology

2.1. Study Area and Sample Collection

Solobo samples and *Lamugin* were collected from different hawkers at different bus terminals marketplace, on the streets in the Accra Metropolis and analyzed to

determine the microbial quality of the locally prepared drink. A total of twenty samples of *Sobolo* (20) and *Lamugin* (10) were procured from various vendors in the afternoon (between 12:30 p.m. and 1:30 p.m.). All samples were immediately placed on ice in Themo's chest and transported to the laboratory for analysis on the same day. To avoid storing samples for long periods of time, three samples (2 *Sobolo* and 1 *Lamugin*) were taken per day. Beverage handling and packaging were also monitored during the selling process. Each sample was assigned a unique study number for easy tracking through the analyses pipeline including standard culture and biochemical techniques for the identification of bacterial and fungal pathogens.

2.2. Bacteriological Analyses

Standard laboratory procedures with strict adherence to aseptic techniques were actively followed. Briefly, each sample was diluted 1:10, (10^{-1}), from which serial dilutions were made further to 10^{-4}. One millilitre of each was pour-plated on pre-labelled plates of Plate Count Agar for Total viable count, incubated at 37˚C for 24 hours. Macroscopic colonies on each plate for the respective dilutions were counted and the number was multiplied by the corresponding dilution factor to obtain the Colony Forming Units per millilitre (CFU/ml) for each plate. The total CFU/ml of a sample was calculated from the four dilutions made from that sample. While plates with relatively few colonies were immediately counted and recorded, plates with many colonies were tallied using the quadrant technique. Plates with too many colonies to count (TNTC) were not considered. Additionally, Blood agar, McConkey agar, and Sabouraud, Dextrose agar was injected to separate fungus, coliforms, and fastidious bacteria, respectively.

To verify the existence of several bacterial pathogens, Gram staining-which determines if bacteria are Gram-positive or Gram-negative based on staining properties-as well as colony morphology and biochemical testing were employed. The Catalase and Coagulase tests were used to identify the Gram-positive cocci bacteria. To identify the specific Gram-negative bacteria, biochemical tests including motility, indole, citrate, urease, oxidase, and fermentation assays were performed.

All identified bacteria were further confirmed using Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS). Manufacturer's instructions on extended direct Transfer method of identification as previously described [16].

To assess the safety level of microbial contamination of the samples, the Food Standards Code for determining the microbiological quality of ready-to-eat foods was used in this study. This code classifies food as acceptable, good, and unsatisfactory based on levels of microbial load in foods (**Table 2**) and previously described [17].

3. Results

Women between the ages of 20 and 29 (66.7%) made up the majority of street

beverage vendors in Accra. Even though 15 of the sellers (or 50%) had never attended school, they nevertheless demonstrated excellent hygiene practices, as seen by the high frequency of hand washing and personal care (Table 3).

Table 2. Levels of recommendation for defining the microbial quality of ready-to-eat food.

	Microbial count in CFU/mL		
Product	Good	Tolerable	Not Satisfactory
Category A	$<10^4$	$<10^5$	$>10^5$
Category B	$<10^6$	$<10^7$	$>10^7$

Key: Category A represents meals that are fully prepared and ready to eat, such as fast-food noodles. Foods that are cooked but then processed further, such as "Sobolo", Lamugin, plantain chips, fall into category B. Foods that aren't prepared, such as chopped fruits, yoghurts, and fresh milk beverages, as well as fermented foods fall under category C.

Table 3. Characteristics of street beverage vendors, Accra, Ghana.

	Parameter	**Frequency (n = 30)**
Age	≤20	7 (23.3%)
	20 - 29	20 (66.7%)
	30 - 39	3 (10%)
Sex	Female	22 (73.3%)
	Male	8 (26.7%)
Education attainment	No education	15 (50%)
	Primary	5 (16.7%)
	Secondary	10 (33.3%)
Personal hygiene	Hands washed at least 3 times daily	18 (60%)
	Hands not washed throughout the day	8 (26.7%)
	Clean cloths	23 (76.7%)
	Fingernails trimmed	7 (23.3%)
	Hair covered	9 (30%)

The target market, beverage production and handling procedures, vending locations, categories of vendors from whom samples were purchased, and hygienic site conditions are provided below (Table 4). Although mobile vendors made up the bulk of vendors (66.7%), most of their clients (60%) were employed individuals. Of the vending venues, only three (10%) were deemed extremely dirty; the others had mostly clean surroundings. A few hours before being moved to the selling locations, all (100%) of the beverages had been prepared in the houses of the individual sellers or their suppliers; not a single beverage had been made there.

Microbial Enumeration and Bacteria identification

The range of microbial loads (total viable count) for *Lamugin* among all ten

Table 4. Characteristics of vending site and customers and Beverage handling practices, Accra, Ghana.

Parameter	Number (%)
Type of Vendor	
Stationary	10 (33.3)
Mobile	20 (66.7)
Type of customers	
Students	12 (40)
workers	18 (60)
Vending Site	
Open air	18 (60)
School	12 (40)
Vending site hygiene	
Litter	3 (10)
No litter	24 (80)
Stagnant water on ground	3 (10)
Mode of preparation	
Beverage is prepared well in advance of consumption.	30 (100)
Beverage scooped into polythene bags.	20 (66.7)
Beverage handled at ground level	20 (66.7)

vendors was 2.21×10^4 - 1.08×10^8 CFU/mL [4.344 - 8.033) log CFU/mL]. The sample from vendor 8 had the least amount of microbial contamination (2.21×10^4, or 4.344 log CFU/mL), while *Lamugin* from vendor 3 had the most (1.08×10^8 CFU/mL, or 8.033 log CFU/mL). On the other hand, except for samples from vendors 5 (2 coliforms) and 6 (1.5 coliforms), the majority of the *Lamugin* samples, including those from vendors 8 and 3, contained no coliforms (zero total coliform counts). Even still, these numbers were insufficient to raise any red flags because they were below the threshold of 30 coliforms. It was also approximated to find the average total viable count for suppliers in each category. The counts were as follows: bus terminal, marketplace, public school, street vendors, and food joint, in decreasing order. Consequently, the food restaurant had the lowest viable numbers and the bus station the highest. However, save from street vendors (5.87 ± 0.01 log CFU/mL) and food joint vendors (5.34 ± 1.04 log CFU/mL), samples from other vendor groups exhibited viable counts that were higher than the acceptable limit of <7 log CFU/mL. Similarly, the study shows that samples from the remaining vendors exceeded the maximum microbiological limit of 7 log CFU/mL after vendors 5, 6, 8, and 10 were excluded.

No matter whether the vendor provided the sample, all *Sobolo* samples were deemed safe for ingestion because their colony-forming units per millilitre were

within the permissible range of 0 - 10^7 CFU/mL [17]. Vendor 4 had the largest microbial load for total viable, with 2.3×10^5 CFU/mL. The sole vendor whose sample showed microbiological growth on the chromogenic coliform agar was vendor 4, a local food vendor in Madina. On the other hand, *Solobo* from seller 9 at an Adenta shopping centre had the lowest CFU/mL, measuring 9.0×10^2 CFU/mL.

Bacillus sp. was identified as gram-positive, spore-forming rods that were also catalase-positive, with flat, uneven edges, and dried colonies. *Klebsiella* sp. were detected as red, mucoid, slightly elevated colonies that were motility-negative, citrate-positive, indole-negative, and Gram-negative on McConkey agar plates. *Pseudomonas* sp. was discovered as gram-negative rods that were also indole-negative, oxidase-positive, citrate-positive, and mucoid colonies with diffusible green pigment. *Staphylococcus aureus* was identified by circular pinhead colonies that were gram-positive, catalase-positive, and coagulase-positive; *Escherichia coli* was determined by red, mucoid, and slightly raised colonies from McConkey plates that showed gram-negative, indole-positive, and citrate-negative reactions. All bacteria identified were confirmed using MALDI-TOF Mass spectrometry.

On Sobolo, a total of 9 fungal species (*Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium oxysporum*, *Fusarium citrinum*, *Fusarium avenaceus*, *Curvularia lunata*, *Penicillium digitatum* and *Rhodotorulla* spp.) belonging to five (5) genera *(Aspergillus*, *Fusarium*, *Curvularia*, *Penicillium* and *Rhodotorula*) were identified (Table 5) from both media.

Seven distinct isolates were identified among the thirty samples examined: *Aspergillus* sp., *Bacillus* sp., *Klebsiella* sp., *Pseudomonas* sp., *Streptococcus* sp., *Staphylococcus aureus*, and *Escherichia coli*. All the samples that were analyzed had a pH value between 2.0 and 5.2. Aspergillus sp. were the most prevalent isolates, as evidenced by their presence in 21 out of 30 unused vials. The next most prevalent isolates were *Enterobacter* spp., followed by *Bacillus* sp., *Escherichia coli Samonella* spp., *Staphylococcus aureus*, *Lactobacillus* sp., *Klebsiella* sp., *Rhodotorula* spp., and *F. verticillodes* which was the least prevalent isolate.

Table 5. Microorganisms isolated.

Beverage	Microorganisms identified
Sobolo	*Escherichia coli*
	Staphylococcus spp.
	Enterobacter spp.
	Salmonella
	Bacillus sp.
	Pseudomonas sp.
	A. fumigutus
	A. niger
	F. verticillodes

Continued

Lamugin	*Escherichia coli*
	Staphylococcus spp.
	Enterobacter spp.
	Salmonella spp.
	Bacillus spp.
	Lactobacillus spp.
	A. fumigutus
	A. niger
	Rhodotorula spp.
	F. verticillodes

4. Discussion

Local beverages such as *Sobol*o, *Lamugin*, and others hold significant cultural and economic importance in Ghana. These drinks, rooted in historical and traditional practices, are not just sources of refreshment but are also deeply intertwined with Ghanaian society's social and cultural fabric

They are produced using locally sourced ingredients, which not only makes them affordable but also accessible to a large portion of the population [1]. This affordability ensures that local beverages play a crucial role in the daily lives of urban residents, providing them with a refreshing and culturally resonant alternative to commercially produced drinks.

As reported in this study, the economic benefits of these drinks are substantially evidenced by the dependence of many vendors on the production and sale of these beverages for their livelihood. The COVID-19 pandemic saw a surge in demand for these drinks, especially those believed to have immune-boosting properties, like *Sobolo*. This increased demand provided economic relief to producers and sellers, showcasing the potential adaptability and resilience of the local beverage market in the face of global challenges [10] [18].

The potential medicinal benefits of local beverages are also highlighted in this study. *Sobolo*, for instance, is for its antimicrobials antioxidant and anti-neuroinflammatory properties [2]. The use of these beverages as alternatives to traditional medicine [19] [20] underscores their importance in the health practices of various ethnic groups in Ghana. For example, the Hibiscus sabdariffa plant, used in making *Sobolo*, is rich in organic acids, phytosterols, polyphenols, and other bioactive substances, making it a beverage with significant health benefits [9]. Such properties are crucial, especially in regions where access to conventional medicine may be limited or unaffordable. Nevertheless, the health benefits of these drinks can be significantly undermined by poor hygiene practices and contamination during preparation. Therefore, while these medicinal properties are beneficial, they must be viewed critically in the context of preparation and consumption.

Despite the cultural, economic and potential medicinal importance of these local beverages, our study raises significant concerns about their safety, particularly due to microbial contamination [13]. The preparation of these beverages, often done in non-sterile environments with minimal regulatory oversight, makes them susceptible to contamination by harmful bacteria and fungi [4]. The impact of this non-supervised production could have been exacerbated by the need to meet the high demand brought about by the Covid-19 pandemic which led some vendors to cut corners on hygiene and safety by resorting to the use of substandard raw materials, unsanitary water, and inadequate storage facilities which could have exacerbated the contamination levels [9]. The quality of local beverages in Ghana is influenced by multiple pollution sources, such as contaminated water sources, poor waste management practices, and unregulated industrial activities. Wastewater that is not properly treated from industrial sources, residential areas, and agricultural runoff poses a risk of contaminating water bodies utilized for beverage production. Moreover, inadequate disposal of solid waste, including plastic bottles and packaging materials, can result in the contamination of water sources and soil. Industrial emissions and inadequate chemical storage present significant risks to the safety of local beverages. The presence of harmful contaminants in beverages can be influenced by these factors, which may impact their quality and safety for consumption.

The presence of microbes such as *Escherichia coli*, *Staphylococcus aureus*, *Shigella* spp., *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* with loads exceeding the safe limits in the samples used for our study conducted in the Greater Accra region of Ghana are comparable a previous report from the Ashanti region of Ghana [4]. These figures are alarming and highlight the potential health risks associated with consuming these beverages. This is because some of these bacteria are known microbes associated with processing of beverages and can cause severe illnesses, including dysentery, and other gastrointestinal infections [17].

Our study highlights acritical issue regarding the regulation of local beverage production in Ghana [18]. The fact that these beverages are often produced in individual homes without any formal oversight or adherence to aseptic guidelines poses a significant challenge to ensuring their standardisation and safety [1] [3]. While the cultural and economic importance of these beverages cannot be overstated, there is an urgent need for improved regulatory frameworks to ensure that they are produced under safe and hygienic conditions. Implementing stricter regulations and providing education to vendors on proper hygiene practices could significantly reduce the risks associated with these beverages. For instance, regular inspections of production sites, enforcement of sanitation standards, and mandatory training for vendors on safe handling practices could help mitigate the contamination risks. Additionally, public awareness campaigns about the potential health risks of consuming poorly prepared local beverages could encourage consumers to demand higher standards from vendors, thereby driving improvements in the market.

5. Conclusion

Local beverages in Ghana, such as *Sobolo* and *Lamugin*, play a vital role in the cultural, economic, and medicinal landscape of the country. However, the potential health risks associated with their production and consumption cannot be ignored. Our study findings underscore the need for improved safety standards and regulatory oversight to ensure that these beverages are safe for consumption.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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