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Bacteriological Evaluation of Selected Food Drinks Commercially Produced in Kumasi in the Ashanti Region of Ghana.

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Abstract

Every day, on average, one million six hundred thousand people get sick due to food, contamination by microorganisms, or chemical substances worldwide. Food safety can also include practical measures and scientific discipline taken to ensure that food products are safe for consumption, free from contamination and do not pose any risk to public health. This research focuses on human pathogenic bacteria for which food is conclusively demonstrated as their transmission mode to humans and the impact of foodborne diseases on public health.

Aim: This study aims to examine and identify common pathogenic bacteria present in locally made food drinks in the Kumasi market.

Methods: Two sites, Kejetia and Abinkyi markets were used for the sampling of the various food products. Convenient sampling was employed in the selection of the food products. A total of eight samples were taken, four from each market. The food products include Pineapple juice, Zonkom, Tiger nut drink, and Brukina. Samples were kept at the same temperature as on the market. The samples from retailers were kept immediately in an ice chest containing ice cubes and were transported to the laboratory. 20 ml of each food product was transferred into a 100 mL sterile container in a 1:5 dilution. A 1-unit sample volume (20 mL) was added to 4-unit volumes of distilled water to produce five units of total volume (100 mL). Temperatures of the various samples were controlled to attain room temperature before subjecting them to culturing.

Results: The results indicate over 62% of the total samples is contaminated with yeast and staphylococcus. Klebsiella sp. was 15% of total samples and Enterobacter species was 7%.

Conclusion: The results show that local drinks have a potential health threat of bacterial pathogens present in these drinks.

Keywords: Brukina, Zonkom, Pineapple Juice, Tiger Nuts, Bacteria, microorganism. Abinkyi, Kejetia.

INTRODUCTION

1.0 BACKGROUND

Globally access to safe and nutritious food is not only recognized as a right in many countries but is a vital part of supporting larger public health goals [1]. Food safety refers to the series of practices anyone preparing food adopts to ensure that the food is safe to eat, and it is the backbone of a trustworthy food

supply chain and supports national economies, trade and tourism [2]. Food safety can also include practical measures and scientific discipline taken to make certain that food products are safe for consumption of mankind, free from contamination and do not pose any risk to public health. The goal of food safety is to prevent consumers from the inauspicious health effects of consuming unsafe or contaminated food [3]. Every day, on average, one million six hundred thousand people get sick due to unsafe food, contaminated with bacteria, viruses, parasites, or chemical substances It affects all countries [4]. The United Nations General Assembly established World Food Safety Day in 2018 to raise awareness of the importance of food safety. The campaign has played a significant impact on food safety in many parts of the world and there is more space to cover [5].

In general, bacterial pathogens cause foodborne diseases by three mechanisms: ingestion of preformed toxins in foods (intoxication; e.g., Staphylococcus aureus), production of toxins within the gastrointestinal tract following ingestion of pathogens (food toxico-infection; e.g., Clostridium perfringens), or invasion of the intestinal epithelial cells (infection; e.g., Salmonella) [6]. Most foodborne bacterial pathogens are often associated with self-limiting gastroenteritis syndrome with nausea, vomiting, diarrhoea, abdominal pain, and sometimes fever. Nevertheless, such bacteria might also cause severe illness with extraintestinal infections, post infection sequelae, and even death, especially in individuals in high-risk groups (infants, young children, the elderly, and immunocompromised patients) [7].

Different microorganisms present in local commercially produced food drinks as contaminants, yet few can develop in the presence of acidic and low oxygen conditions [8]. Notably, yeast is the dominant microbial group present in fruit juices and other drinks. Bacterial, yeast and moulds are vital microbes which cause deterioration and food poisoning [9]. The primary reason for the deterioration in food drinks is because of the contamination caused by bacteria, fungi, yeast, and sometimes damage caused by insects [9, 10].

Microbes confer favourable physicochemical and biological properties and improve the taste, aroma and shelf life of products at very low cost. Microorganisms also contribute to cost savings and revenue manufacture within the food industry [11, 12]. Due to the increasing demand to produce food products with desirable sensory quality, long shelf life and containing natural ingredients, fermented products have been given attention to meet the needs of consumers [12].

The Industrial Revolution brought very noticeable changes in the production of food and its distribution. Along with the growth of urban populations and mass production, concerns about food safety escalated. In the mid-19th century, scientists like Louis Pasteur and Robert Koch made groundbreaking discoveries about the role of microorganisms in food spoilage and illness, laying the foundation for modern food microbiology and safety practices [13]. The 20th century witnessed the establishment of regulatory authorities such as the World Health Organization (WHO) and Food and Drug Authorities which played crucial roles in setting food safety standards and regulations on a global scale. Advances in food science, technology, and inspection methods have allowed for more effective monitoring of food safety management systems like Hazard Analysis and Critical Control Points (HACCP) [14]

During the manufacturing of these commercial food drinks, many microbes have been involved, and a few will be a reason for causing spoilage in it and subsequently food poisoning [15]. The quality of

sensory will be degraded with the spoilage and leading to changes in visual, odour, and flavour changes. Microbial growth must reach a certain quantity to spoil the beverages [9]. Secondary metabolite from the microbe will also lead to indirect spoilage other than microbial growth. If the raw materials are contaminated, that will lead to the product's spoilage, failure in the production process, higher production of foam, and loss of flavours [9, 16]. Food preservation is the process of extending the shelf life of food by inhibiting the growth of microorganisms, enzymes, and other factors that can cause food spoilage. Preservation methods can be physical, chemical, or biological. Physical preservation methods include refrigeration, freezing, drying, and irradiation. Refrigeration and freezing slow down the growth of microorganisms and enzymes while drying and irradiation eliminate them by removing or destroying the water needed for their growth [17]. As different microorganisms prefer different environments for growth, a specific food drink will enhance a microbe even if the products are manufactured in better production conditions [8, 9]. In northern Ghana and particularly the central market of Kumasi in Ghana, the most famous and preferred locally made beverages are tiger nuts drink, sobolo, pineapple juice, zonkom, mashed kenkey, Brukina, etc. The main problems associated with these products, however, are the preparation process, source of material and their short shelf-life when they are kept at ordinary room temperatures because of activities of microorganisms which actions are facilitated by a conducive environment [18]. Foodborne illnesses, also known as food poisoning or foodborne diseases, are major health problems that are caused by the usage of contaminated food. These illnesses can result from various biological agents, including bacteria, viruses, parasites, and fungi [19].

Food is considered one of the main environmental drivers shaping the human microbiota across the lifespan. Microorganisms vehiculated by food can be related to a variety of scenarios, including those benefiting health (e.g., stimulation of host antibodies, the release of chemicals to stimulate the health of the overall system or inhibition of pathogen development), those causing minimal change within the equilibrium of the host microbial community, and those that are pathogenic or have been associated with gut-host dysbiosis [20]. Recently there has been an increase in knowledge on gut bacterial genera and species commonly affected by diet, as well as evidence suggesting that the intestinal microbiome plays an important role in modulating the risk of several chronic diseases (e.g., inflammatory bowel disease, obesity, type 2 diabetes, cardiovascular disease, and cancer [21, 22].

1.1 PROBLEM STATEMENT

Bad production practices and unhygienic sources of raw materials and conditions exposed to the products, implicate in food and drinks harmful substances and bacteria which are consumed resulting in food poisoning or illness with acute gastroenteric action as a major symptom.

Ghanaians over the years have come to accept locally prepared and manufactured food drinks hawked on the streets and markets of Kumasi Metropolis as perceived nutritious due to their composition. Some Communities in the Kumasi Metropolis have also jumped onto these food drinks due to their sweetness and affordability. Despite the benefits, the product tends to have minimal shelf life. The spoilage of these drinks that are consumed by many is because of microbial activities which gradually occur over time. The activities of these microbes must reach certain levels to manifest contamination of these products, threatening potential food poisoning which is detrimental to public health. Subsequently, certain conditions such as temperature, sugar content, acidity and water necessitate the growth or the activities of bacteria in these manufactured or prepared drink products. This research will focus on human

pathogenic bacteria for which food is conclusively demonstrated as their transmission mode to humans and the impact of foodborne diseases on public health. The implication of food chain (foodborne pathogens and commensals) in the transmission of resistance to antibiotics relevant to the treatment of human infections is also evident. Since these products sometimes are not subjected to any quality control or assurance procedures, it becomes difficult to tag a proper expiry date to these products and only recognize unfit to drink or use until there is a change in colour, taste and moulds. Due to various volumes, ingredients and exposed conditions, the microbial activity and levels needed to cause spoilage of these products may vary. In 2015, DALY's data showed the loss of 33 million healthy lives, most among children less than 5 years of age and in low-income countries (African and Southeast Asian regions) due to food-borne diseases. The most frequent causes of foodborne disease worldwide are bacterial pathogens, the most important being the zoonotic Campylobacter and nontyphoidal Salmonella (NTS). Certain diseases, such as those caused by NTS, are a public health concern across all regions of the world, in high- and low-income countries. In human health a single microbe can cause a very devastating condition which may results in decreasing the health quality of people within a locality, therefore it is imperative to evaluate bacterial content of these drinks to inform proper policies, minimize potential food poisoning and improve the quality of health of the people.

1.2 Aim

This study aims to examine and identify common pathogenic bacteria present in locally made pineapple juice, zonkom, tiger nut drink and Brukina in Kejetia and Abinkyi markets in Kumasi.

1.3 Specific Objectives

The specific objectives of the study are to:

1. determine the pH of selected locally made food drinks.
2. identify pathogenic bacteria present in locally made food drinks.
3. enumerate pathogenic bacteria identified in locally made pineapple juice, zonkom, tiger nut drink, and Brukina.

1.4 Justification

Since these products sometimes are not subjected to any quality control or assurance procedures, it becomes difficult to tag a proper expiry date to these products and only recognize unfit to drink or use until there is a change in colour, taste and moulds. Due to various volumes, ingredients and exposed conditions, the microbial activity and levels needed to cause spoilage of these products may vary. The most frequent causes of foodborne disease worldwide are bacterial pathogens, the most important being the zoonotic Campylobacter and nontyphoidal Salmonella (NTS). Certain diseases, such as those caused by NTS, are a public health concern across all regions of the world, in high- and low-income countries.

Materials and Methods

Indigenous hawked food drinks; Pineapple juice (PJ), Zonkom (ZK), Tiger nut drink (TD), and Brukina (BRK).

Culture media

The following culture media were used in the practical section of the project; blood agar, chocolate agar, MacConkey agar and XLD agar

Reagent

Distilled water

Glassware and equipment

Sterile containers, Incubator, measuring cylinder or beaker, Ice cheers, pH Meter and Petri dish.

Sample collection and preparation

Two major sites were used for the sampling of the various food products. These were the Kejetia market and the Abinkyi market, all in Kumasi, the Ashanti regional capital. Convenient sampling was employed in the selection of the food products from the two main sites. Four different types of drinks were taken as samples for the study. A total of eight (8) samples were taken, four from each market.

The food products were; Pineapple juice, Zonkom, Tiger nut drink and Brukina. For easy identification, the alphabets A and B were suffixed to the codes to denote products from Abinkyi market and Kejetia market respectfully.

Sample processing

Samples were kept at the same temperature as on the market. The samples from retailers on the market were kept immediately in an ice chest containing ice blocks and were transported to the lab. 20 mL of each food product was transferred into a 100ml sterile container. In a 1:5 dilution, 1-unit volume of sample (20 mL) is combined with 4-unit volumes of distilled water (solvent) to produce five units of total volume (100 mL). Temperatures of the various samples were controlled to attain room temperature before subjecting them to culturing.

Culturing Procedure

1. Sterile Petri dishes containing culture media were labelled accordingly.
2. Diluted samples were poured on culture media on a petri dish.
3. Samples were then cultured on blood agar, chocolate agar, MacConkey agar and XLD agar
4. Samples cultured on the agar were incubated at 37^{o}C overnight (18-24 hours). This incubation was aerobic.
5. Upon inspection, plates with no growth were reported as “No Bacterial Growth” and plates with bacterial growth were subjected to identification methods.

Bacteria Identification and Enumeration.

Specific agar for culturing bacteria was employed in targeting the growth of bacteria of interest. Morphological observation of the colony was adopted throughout the identification of bacteria growth.

None of the cultures was anaerobically incubated. Also, colony count of the various isolated bacteria was not feasible because of the polymicrobial nature of the samples. It was partly also due to the use of a non-calibrated loop for the culturing of the samples onto the culture media.

Blood agar in some cases facilitates the growth of fastidious organisms and is a differential base on the hemolytic property of the bacteria. Streptococcus species present appear transparent or produce green colouration around the colony. Chocolate agar is for the isolation of both aerobic and anaerobic organisms. Chocolate agar is also an enriched medium for the isolation and identification of fastidious organisms. MacConkey is selective and differential and only grows gram-negative bacterial species. It can differentiate gram-negative bacteria based on their lactose metabolism. Staphylococcus spp. and Enterobacter spp. are identified on MacConkey media. Salmonellae spp. and Shigellae spp. grow in Xylose lysine deoxycholate media.

Measuring pH

Samples were also tested for pH and recorded at room temperature (27℃).

Results and Discussion

Results

Figure 1 represents the prevalence of Klebsiella spp, Staphylococcus spp, Enterobacter spp, Salmonella spp and Bacillus spp in samples selected from two different markets.

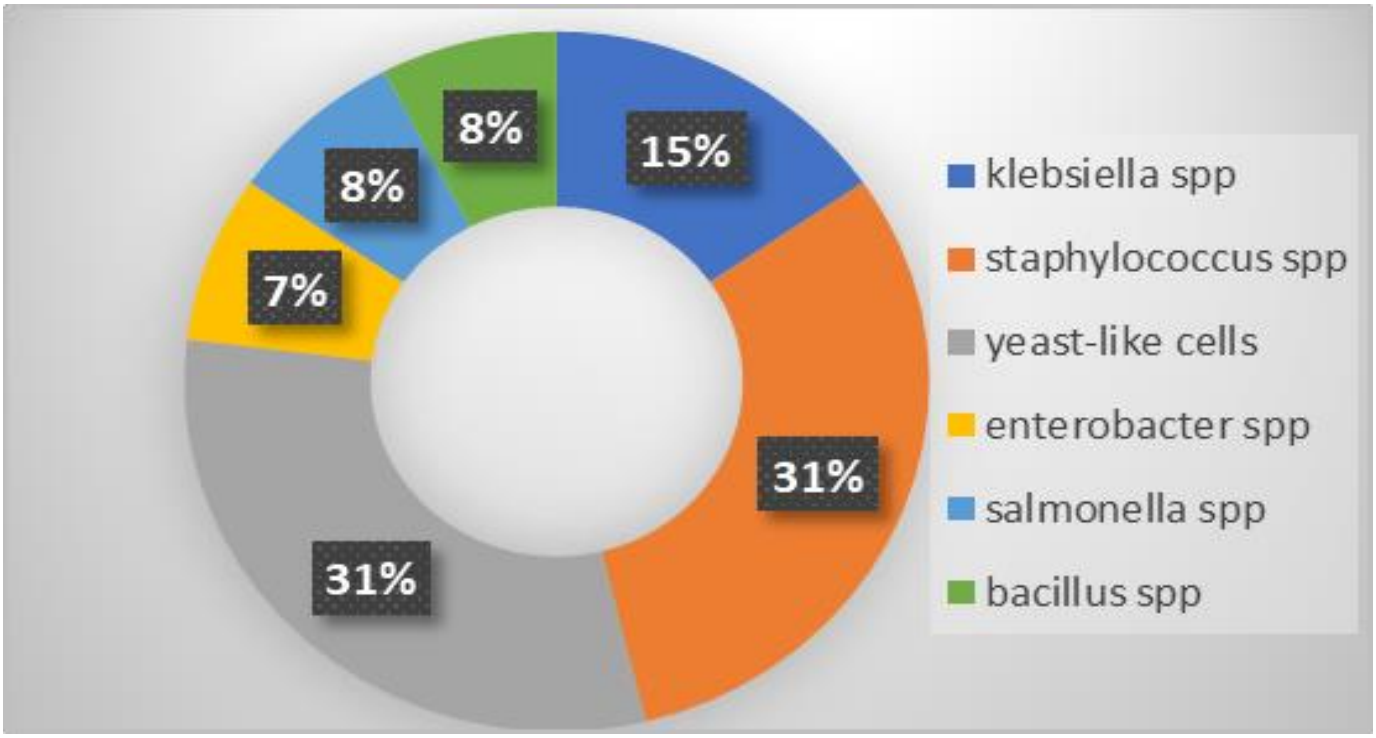

Fig. 1 The prevalence in the percentage of bacteria found in entire samples collected on the field.

The result indicates over 62% of the total samples were contaminated with yeast and Staphylococcus spp. Yeast takes up 31% and Staphylococcus spp also picks up 31% of the total food samples. Since these products contain no preservatives, it's expected to go bad in a short period or when not kept in the appropriate condition. It is therefore likely these products might start going bad before consumption. Klebsiella spp. picks up 15% of total samples and Enterobacter spp. with the least appearance in samples recording 7%.

Table 1. reports the observations made after culturing samples. Bacteria identification by morphological characteristics including colony morphology such as colour, size, shape, opacity, elevation, margin surface texture and consistency.

MPLE	CODE	OBSERVATION ON MEDIA	INFERENCE
Pineapple Juice	PJ-A (A-ABINKYI)	Blood agar: **X** MacConkey: Large mucoid and a diffusing red Chocolate agar: **X** XLD agar: **X**	Klebsiella spp
Pineapple Juice	PJ-B (B-KEJETIA)	Blood agar: **X** MacConkey agar: Pink to red colony and a tiny red colony Chocolate: **X** XLD agar: **X**	Staphylococcus spp Enterobacter spp.
Zonkom	ZK-A (A-ABINKYI)	Blood agar: **X** MacConkey agar: Pink to red colony Chocolate agar: **X** XLD agar: **X**	Yeast-like cells (Fungal element) Staphylococcus spp.
Zonkom	ZK-B (B-KEJETIA)	Blood agar: **X** MacConkey agar: Pink to red colony Chocolate agar: **X** XLD agar: **X**	Yeast-like cells (Fungal element) Staphylococcus spp.
Tiger Nut Drink	TD-A	Blood agar: **X** MacConkey agar: Pink to red colony and	

	(A-ABINKYI)	large mucoid with diffusing red Chocolate agar: **X** XLD agar: **X**	Staphylococcus spp Klebsiella spp
Tiger Nut Drink	TD-B (B-KEJETIA)	Blood agar: **X** MacConkey agar: Chocolate agar: **X** XLD agar: Colony with black enter	Bacillus spp. Salmonella spp.
Brukina	BRK-A (A-ABINKYI)	Blood agar: MacConkey agar X Chocolate agar: **X** XLD agar: **X**	Yeast-like cells (Fungal element)
Brukina	BRK-B (B-KEJETIA)	Blood agar: MacConkey agar: **X** Chocolate agar: **X** XLD agar: **X**	Yeast-like cells (Fungal element)

Table 1. Bacteria identification by colony morphology.

X – No bacteria identified

Table 2 represents the bacteria species identified and the corresponding pH of samples.

SAMPLE	**SITE**	**CODE**	**ISOLATE(S)**	**pH**
Pineapple Juice	Abinkyi Market	PJ-A	1. Klebsiella spp	3.4
Pineapple Juice	Kejetia Market	PJ-B	1. Staphylococcus spp 2. Enterobacter spp	3.1
Zonkom	Abinkyi Market	ZK-A	1. Yeast-like cells (fungal elements) 2. Staphylococcus spp	8.1

Zonkom	Kejetia Market	ZK-B	1. Yeast-like cells (fungal elements) 2. Staphylococcus spp	7.8
Tiger Nut Drink	Abinkyi Market	TD-A	1. Staphylococcus spp 2. Klebsiella spp	4.6
Tiger Nut Drink	Kejetia Market	TD-B	1. Bacillus spp 2. Salmonella spp	4.9
Brukina	Abinkyi Market	BRK-A	1. Yeast-like cells (fungal elements)	4.2
Brukina	Kejetia Market	BRK-B	1. Yeast-like cells (fungal elements)	3.8

Table 2: Bacteria observed after culture with respective sample pH

DISCUSSION

Most of the time consumers are not conscious about the safety, quality and hygiene of locally produced drinks. It can be a potential factor for food poisoning or food-borne diseases. Because of the threat posed and the flourishing demand for these drinks, the study aimed at identifying common pathogenic bacteria consumed in local commercially produced food drinks on Kumasi markets. The acidity and alkalinity of these food drinks were considered, anticipating their effect on the growth and presence of bacteria. None of the cultures was anaerobically incubated. Also, colony count of the various isolated bacteria was not feasible because of the polymicrobial nature of the samples. The pH of all the food samples was acceptable as other studies show similar ranges. The low pH of fruit juices greatly limits the number and the type of bacteria that can survive or grow at that Ph [23, 24, 25, 26].

After 24 hours of incubation, pink to red colonies on MacConkey agar were primarily considered Staphylococcus spp. Colorless, transparent, with a black centre colony in XLD agar considered as Salmonella spp. large mucoid with a diffusing red presence on MacConkey agar was recorded as Klebsiella spp. The compact tiny red colony on the plate was also registered as Enterobacter spp.

The least contamination of bacteria occurred in Brukina which had the growth of yeast cells. Yeast has a positive role in the fermentation of some products and is also responsible for food spoilage. Taking into consideration, the milky nature of the product it might indicate the spoilage of the product even before consumption.

The presence of Staphylococcus spp. Salmonella spp. and Klebsiella spp. in some of the products imply a negative relation with food quality and safety.

But to prevent such contamination in locally produced drinks on markets, good hygienic production principles and quality raw material sources would contribute to the basis for safe food production. The

government health agencies must adopt measures to ensure the safe production of locally produced drinks and develop a consistent monitoring system.

Geographical effect on bacteria contamination

In most cases, product contamination, results from the source of raw materials, in-process contamination and storage of finished products. Taking into consideration, the filthy nature of our marketplaces, this research decided to check whether there could be a possible contamination from the market. The results do not depict any possible contamination from the different marketplaces as product contaminants appear to be the same. The contamination or presence of these potential pathogenic bacteria might come from the source of raw material or in-process contamination by personal or environmental hygiene.

CONCLUSION AND RECOMMENDATION

Conclusion

Nutritious and availability make locally produced food drinks on our markets more patronized by the people. These drinks would have achieved their full intended purpose if they were free from pathogenic bacterial contamination. It is therefore imperative to regularize the production of these local drinks as this study shows a potential health threat of bacterial pathogens present in these drinks.

Recommendation.

1. Further research on bacterial enumeration on these products.
2. Employment of another bacterial identification method (biochemical test) to confirm specific species or strains present in these food products.
3. Government health agencies must adopt measures to ensure the safe production of locally produced drinks and develop a consistent monitoring system.
4. Anaerobic culturing techniques should be employed.

Ethical Committee Approval was given

Limitations: The study could not cover all the markets in Kumasi city.

Anaerobic culturing techniques could not be employed

Authors Contribution

1. Egote Alexander Kofi, manuscript writing and editing
2. Asumadu Prince, data collection.
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7. Cardinal Newton, administrative support

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Data Availability Statement:

Study data is available and will be provided by the authors upon request.

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