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# Microbiological Quality of a Locally Brewed Alcoholic Beverage (PITO) Sold in Prampram within the Greater Accra Region, Ghana

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# Authors' contributions

This work was carried out in collaboration between all authors. Authors AAM, EA and PBTQ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EA, ALM and PBTQ managed the analyses of the study. Authors ENL and AAM managed the literature searches. All authors read and approved the final manuscript.

# Article Information

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**Original Research Article** 

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# ABSTRACT

**Aims:** This study evaluated the microbiological quality of a locally brewed alcoholic beverage (pito). Therefore, bacteria and fungi present in the pito samples were examined. **Study Design:** This was a cross sectional study.

**Place and Duration of Study:** Department of Science Laboratory Technology, Accra Technical University, spanning from March to mid-April 2016.

**Methodology:** Samples of the drink were collected every week for six weeks, from three different popular brewing sites at Lower Prampram in the Ningo-Prampram District of Accra, Ghana. The samples were processed and examined for bacteria and fungi using the Standard Plate Count (SPC) technique.

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**Results:** A total of six different bacteria and a fungus were isolated. The bacteria were *Escherichia coli, Klebsiella pneumoniae, Shigella* spp, *Enterobacter aerogenes, Staphylococcus aureus* and *Pseudomonas aeroginosa,* whiles the fungus was *Saccharomyces cerevisiae*. Total viable counts as well as individual isolates counts in all the pito samples were found to be less than 10<sup>4</sup> CFU/ml. **Conclusion:** It is noteworthy that, *Saccharomyces cerevisiae,* the only fungus isolated is known to be associated with fermentation and the microbes isolated from the pito samples were found to be within the permissible limits. However, these potentially pathogenic microbes, if found in unacceptable limits, from the fermenting samples could merit public health attention. Therefore, periodic screening of pito and their brewers, coupled with education on the maintenance of recommended guidelines concerning food and drink production is encouraged.

Keywords: Pito; beverage; bacteria; coliform; lower prampram; Ghana.

# ABBREVIATIONS

S. aureus: Staphylococcus aureus, E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa, E. aerogenes: Enterobacter aerogenes, K. pneumoniae: Klebsiella pneumoniae, S. cerevisiae: Saccharomyces cerevisiae, XLD: Xylose-Lysine-Desoxycholate, EMB: Eosin Methylene Blue, CLED: Cystine Lactose Electrolyte Deficient, DRBC: Dichloran Rose Bengal Chloramphenicol, WHO: World Health Organization, F.A.O: Food and Agricultural Organization, ICMSF: International Commission on Microbiological Specifications for Foods, Ni: Nickel, Pb: Lead, Cd: Cadmium, Na: Sodium, K: Potassium, Fe: Iron, Cu: Copper, Zn: Zinc.

# **1. INTRODUCTION**

Pito is a traditionally brewed alcoholic beverage. It is produced mainly from the grains of guinea corn (Sorghum vulgare or Sorghum bicolor) and millet [1]. Pito brewing originally started in the northern part of Ghana but its production is now widely spread over the entire country, serving as a lucrative business particularly for the rural folks. It is produced traditionally by malting, mashing, fermentation and maturation of the grains which are steeped in water over night, after which excess water is drained. The grains are then placed in layers and germinated with periodic moistening. Germination continues for four to five days until the plumule attains a length of about 2.5 cm or longer [2]. The malted grains are spread out in the sun to dry for one to two days after which the dried malt is milled into powder. Boiling of the wort is done among other reasons, to denature malt enzymes and enzymes supplements [2-4]. Pito is golden yellow to dark brown in colour, with taste varying from slightly sweet to sour. It has been observed that, pure cultures of microorganism can be used to reduced fermentation time and improve production process of the pito [5]. Such pure cultures include Lactobacillus plantarum in combination with Saccharomyces cerevisiae and Pediococcus halophilus in combination with Candida tropicalis [5]. The pH, colour, titrable acidity, alcohol content, specific gravity, taste

and flavour of 'Pito' produced by use of pure culture can be compared favourably with that produced using the traditional method [5]. In Ghana, about four types of pito can be identified depending on their wort extraction and These include the fermentation methods. Nandom, Kokomba, Togo, and Dagarti Pito [6]. Compared with European beers, pito is heavier and darker, but less bitter. It is also lighter in colour and thinner in consistency than European stout beer [7]. It is mostly neither bottled nor canned [8], but purchased directly from the household in which it is brewed. It is typically served in a calabash or bottle outside the producer's home where benches are provided for the consumers to sit on and enjoy their beverage. Meanwhile, it has been observed that this beverage can be processed, bottled and stored for 2 months with little or no effect on its characteristics and gualities [9]. Nevertheless, fresh samples of pito have been accepted as better than the stored ones in terms of both microbiological and nutritional quality [10,11].

Pito is usually consumed as a nutritious beverage to quench hunger and thirst. It has social and economic significance, particularly to the people of the three northern regions of Ghana [7]. It is used for the performance of certain traditional functions such as pouring of libations at social gatherings, funerals, marriage and child-naming ceremonies. While pito seems

to serve as refreshment during leisure hours and constitutes a source of income for the producers in the rural areas, when overused intoxicates and could lead to violence as well as indecent behavior as sometimes observed, especially in such large gathering. In addition, all the commonly associated health risks with overconsumption of alcoholic beverages [12] also apply to the misuse of pito. Similar to other openly sold foods and drinks, several factors mav make pito prone to microbial contaminations. Potential microbes that can contaminate pito may include bacteria such as E. coli, Salmonella species, Shigella species and Staphylococcus aureus [13], whiles fungal contaminants may include Aspergillus flavus, Aspergillus niger and Saccharomyces cerevisiae [14-16]. Meanwhile, Saccharomyces cerevisiae is known to be associated with fermentation [14,15,17]. A microbiological and physicochemical analysis of a traditional sorohum beer "Ikigage", collected from the southern province of Rwanda showed the presence of microbes such as aerobic mesophilic bacteria, lactic acid bacteria, coli, Е. fecal streptococci, Staphylococcus aureus, yeast, and moulds [18]. Other contaminant such as aflatoxin can also be associated with such locally produced beverages. For example, in a small market survey, aflatoxin B1 was detected to be associated with a beverage 'Horchata' derived from tiger nuts frequently consumed in Southern Europe [19]. Similarly, aflatoxins were detected in all the samples of traditional opaque sweet beverage (thobwa) and beer prepared from sorghum malts, collected from the southern region of Malawi and analyzed [20]. In that study, the average aflatoxin content in the beer was found to be higher than the permissible maximum level expected to be detected in a ready to eat foods [20].

In most parts of Africa, the passion for drinking outweighs other considerations such as safety of the drinks and beverages, leading to considerable rise in drink-borne intoxications and contamination [21]. A good search through pertinent literature revealed that, not much work has been done to ascertain the microbial quality of pito brewed at Lower Prampram in the Ningo-Prampram district of Ghana, where pito brewing is gaining much prominence and the patronage of the drink is high among the youth. This study therefore, sought to provide information on the microbial quality of this beverage by isolating and identifying microorganisms in pito produced and sold within this community.

#### 2. MATERIALS AND METHODS

#### 2.1 Study Site and Sample Collection

This was a cross sectional study. Three different sites at Lower Prampram in the Ningo-Prampram district of Accra, Ghana, where pito brewing is gaining much prominence were selected for the current study. The sites were Prampram Keba shoo, Prampram Alata and Prampram Abia. Thirty (30) pito samples were bought from consented vendors at the three different sites for a period of six (6) weeks (Spanning from March to mid-April). Within the first 2 weeks, pito samples were bought from ten (10) vendors at one site, after which the samples were prepared and examined at the laboratory. Subsequently, the same process was repeated for the two other sites in the second and third 2 weeks. Prior to the purchase of the samples. verbal interviews and brief discussions were held with the vendors to have an idea of the condition of the places where the pito was brewed and when it was prepared. An observation was also made on how the pito was handled during the selling process. The samples were put in clean, autoclave-sterilized bottles and transported on ice packs to the Microbiology Laboratory of the Department of Science Laboratory Technology, Accra Technical University, from where clearance was obtained, for examination and analysis.

#### 2.2 Serial Dilution of Pito Samples

Stock solutions of the various samples were prepared by pipetting 10 ml each into three sterilized bottles filled with 90 ml sterile peptone physiological saline solution and homogenized. To prepare the diluents, 15 g of peptone powder was dissolved into 1000 ml sterile distilled water. boiled on a hot-plate for peptone to dissolve completely; then sterilized in an autoclave at a temperature of 121°C for 15 minutes. Exactly 9ml of the peptone water was pipetted into each of 9 sterilized test-tubes. A 1ml aliquot of stock sample A was aseptically transferred into one of the nine (9) test-tubes containing 9ml of the peptone solution and mixed thoroughly using whirl shaker to form  $10^{-1}$  dilution. One milliliter (1) ml) of the 10<sup>-1</sup> dilution was aseptically transferred after homogenization into the second test tube containing 9ml of the peptone solution to form 10<sup>-2</sup>. Similarly, 10<sup>-3</sup> dilution was prepared for sample A. This procedure was repeated for stock samples B and C. The samples were serially diluted to reduce the concentration of cells to

more usable concentration in order to obtain a countable plate and more manageable results.

#### 2.3 Culturing, Enumeration and Isolation of Bacteria and Fungi

The conventional Pour Plate method [22] was used in culturing, enumeration and isolation of bacteria and fungi. Briefly, serial diluted samples (10<sup>-3</sup>) were well mixed and using a micropipette with disposable tips, one milliliter (1 ml) aliquot of each diluted sample was pipetted into the center of appropriately labeled empty petri dish. About 15 mL of 45℃ molten medium was poured into the inoculated petri dish. Media used were Plate Count Agar, Xylose-Lysine-Desoxycholate Agar, Cystine Lactose Electrolyte Deficient (CLED) Agar. Eosin Methylene Blue (EMB) agar and Dichloran Rose Bengal Chloramphenicol (DRBC) agar. The media and sample in the petri dish were mix thoroughly by tilting and swirling the dish whiles carefully watching out not to slop the agar over the edge of the petri dish. The agar was then allowed (for about 10 minutes) to completely gel without disturbing it, after which the plate was incubated. All the media and media ingredients such as peptone used in the study were from Scharlau, Spain and Sigma Chemical Co. Ltd., USA. Total viable bacterial count was performed on Plate Count Agar (LAB 149), similar to Lyumugabe et al. [18]. Xylose-Lysine-Desoxycholate Agar (XLD) was used for isolation and enumeration of Klebsiella pneumoniae, Enterobacter aerogenes and Shigella spp., Eosin Methylene Blue (EMB) agar for total coliform and Escherichia coli. Cystine Lactose Electrolyte Deficient (CLED) Agar was used for Staphylococcus aureus and Pseudomonas aeruginosa and Dichloran Rose Bengal Chloramphenicol (DRBC) was used for fungal isolation. DRBC was used because, in addition to oxytetracycline glucose yeast extract agar and rose bengal chloramphenicol agar, it has been identified as being superior to acidified potato dextrose agar for enumeration of yeasts and moulds [23]. Petri plates for bacteria were incubated at 37°C for 24-48 hours whilst fungal plates were incubated at 28°C for 5 days. The colonies seen based on colonial morphology such as colour, shape, size and consistency were counted using a colony counter (Stuart Scientific, UK), and recorded as colony forming unit per millilitre (CFU/ml). The enumeration was carried out in double and the plates containing 30-300 colonies were considered [18]. Bacterial colonies were further sub-cultured onto fresh Agar plates using a sterile loop (2 mm inside

diameter) to obtain pure cultures for further identification. Similarly, fungal colonies were subcultured onto fresh Dichloran Rose Bengal Chloramphenicol (DRBC) Agar to obtain pure culture for further identification (Fig. 1). The plates containing DRBC for fungal isolation were incubated in the inverted position. This method of incubation completely obviated the usually sprinkling of powdery spores of *Penicillium* and *Aspergillus* species on plates incubated in the upright position [24].

# 2.4 Identification of Isolates

Bacterial identification was done based on a number of procedures including microscopy, Gram staining, oxidation-fermentation tests and a battery of biochemical tests such as urease test, catalase test, citrate utilization test, indole test and triple sugar iron test [25-27]. Fungal identification was done using the fungi conventional identification manual [28].

# 2.5 Statistical Analyses

Data obtained was stored in Microsoft Excel and analyzed using the Statistical Package for the Social Sciences (SPSS), version 12.0.1. Data was summarized by determining the means, median, minimum and maximum values of the microbial loads. Association between the study variables was determined. A P-Value less than 0.05 was considered statistically significant.

# 3. RESULTS

Six bacteria namely: *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Shigella* spp. and *Klebsiella pneumoniae* were isolated from all the eighteen (18) samples collected from the three sampling points. *Saccharomyces cerevisiae* was the only fungus isolated (Fig. 1).

All the bacterial and fungal isolates were present at each of the sampling locations (Table 1). The isolates occurred in varied percentages in the samples from each of the sampling points (Table 1). Saccharomyces cerevisiae and Staphylococcus aureus were the highest occurring microbes isolated at Prampram Keba shoo (22.2%) whiles Pseudomonas aeruginosa was the highest (25.0%) among the isolates at Prampram Alata (Table 1). At Pampram Alata, again, Saccharomyces cerevisiae and Enterobacter aerogenes both showed the highest occurrence (22.2%). Meanwhile, Klebsiella

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*pneumoniae* was the least occurring isolate from Prampram Keba Shoo (3.7%), while *Klebsiella pneumoniae* exhibited the least occurrence from Pampram Alata (7.1%). *Shigella* spp and *Escherichia coli* both showed the least occurrence (7.4%) at Prampram Abia (Table 1).

Occurrence ranges of the bacteria from the sampling sites were between 3.7 % - 22.2% for

Prampram Keba Shoo, 7.4% - 22.2% for Prampram Abia and 7.1% - 25.0% for Pampram Alata. Overall, *S. cerevisiae* recorded the highest percentage occurrence among all the microbes isolated with a mean value of 20.8% followed by *Staphylococcus aureus* with a mean value of 18.3%. The least mean percentage occurrence (7.3%) was recorded by *Klebsiella pneumoniae* (Fig. 2).



Fig. 1. Saccharomyces cerevisiae colonies on DRBC agar. (a) Pink and reduced size pure colonies (b) Zoom in of single colonies picked for further identification

Table 1. Number and percentage occurrence of bacteria and fungus isolated from the Pito					
samples					

Sampling sites	Microorganisms	n (%)	
Prampram Keba Shoo (N=27)	Staphylococcus aureus	6 (22.2%)	
	Escherichia coli	4 (14.8 )	
	Enterobacter aerogenes	3 (11.1 1%)	
	Pseudomonas aeruginosa	4 (14.8 %)	
	Shigella spp.	3 (11.1 %)	
	Klebsiella pneumoniae	1 (3.7 %)	
	Saccharomyces cerevisiae	6 (22.2%)	
Pampram Alata (N=28)	Staphylococcus aureus	5 (17.9 %)	
	Escherichia coli	3 (10.7 %)	
	Enterobacter aerogenes	3 (10.7 %)	
	Pseudomonas aeruginosa	7 (25. 0%)	
	Shigella spp.	3 (10.7 %)	
	Klebsiella pneumoniae	2 (7.1 %)	
	Saccharomyces cerevisiae	5 (17.9 %)	
Prampram Abia (N=27)	Staphylococcus aureus	4 (14.8 %)	
	Escherichia coli	2 (7.4 %)	
	Enterobacter aerogenes	6 (22.2 %)	
	Pseudomonas aeruginosa	4 (14.8 %)	
	Shigella spp.	2 (7.4 %)	
	Klebsiella pneumoniae	3 (11.1 %)	
	Saccharomyces cerevisiae	6 (22.2 %)	

N represents the total number of microbes at a particular site. n represents number of particular isolate identified



Fig. 2. Mean percentage occurrence of the bacteria and fungus isolated

The microbes isolated in all the pito samples were found to be within counts of less than  $10^4$  CFU/ml. Different loads of the microorganisms were recorded for each of the individual samples obtained from ten (10) places at a particular location. In the ten samples taken from Prampram Keba Shoo, the count ranges of the microorganism were as follows: *E. coli* (2.2 x  $10^2$  - 2.5 x  $10^2$ ), *S. aureus* (3.5 x  $10^2$  - 3.8 x  $10^2$ ), *Shigella* spp. (1.9 x  $10^2$  - 2.4 x  $10^2$ ), *K. pneumoniae* (0.9 x  $10^2$  - 1.2 x  $10^2$ ), *E. aerogenes* (1.7 x  $10^2$  - 2.1 x  $10^2$ ), *P. aeruginosa* (1.8 x  $10^2$  - 2.4 x  $10^2$ ), Total coliform (1.3 x  $10^2$  - 1.5 x  $10^2$ ), Total viable bacteria (5.2 x  $10^3$  - 5.7 x  $10^3$ ) and *S. cerevisiae* (3.1 x  $10^2$  - 3.7 x  $10^2$ ).

For Prampram Alata, the count ranges of the microorganism were as follows: E. coli (1.5 x 10<sup>2</sup> - 2.3 x  $10^2$ ), S. aureus (3.0 x  $10^2$  - 3.3 x  $10^2$ ), Shigella spp.  $(2.0 \times 10^2 - 2.4 \times 10^2)$ , K. pneumoniae (1.2 x  $10^2$  - 1.6 x  $10^2$ ), E. aerogenes (1.2 x 10<sup>2</sup> - 1.7 x 10<sup>2</sup>), *P. aeruginosa* (3.7 x 10<sup>2</sup> - $3.9 \times 10^2$ ), Total coliform (3.1 x  $10^2$  -  $3.5 \times 10^2$ ), Total viable bacteria  $(3.1 \times 10^3 - 3.4 \times 10^3)$  and S. cerevisiae (2.3 x 10<sup>2</sup> - 3.0 x 10<sup>2</sup>). For Prampram Abia, the count ranges for the 10 sites were: E. coli (1.2 x  $10^2$  - 1.9 x  $10^2$ ), S. aureus (2.1 x  $10^2$  -2.8 x  $10^2$ ), Shigella spp.(1.5 x  $10^2$  - 1.9 x  $10^2$ ), K. pneumoniae (1.9 x 10<sup>2</sup> - 2.2 x 10<sup>2</sup>), E. aerogenes (2.8 x 10<sup>2</sup> - 3.1 x 10<sup>2</sup>), *P. aeruginosa* (2.4 x 10<sup>2</sup> -2.6 x  $10^2$ ), Total coliform (4.1 x  $10^2$  - 4.3 x  $10^2$ ), Total viable bacteria  $(3.1 \times 10^3 - 3.4 \times 10^3)$  and S. cerevisiae (3.0 x 10<sup>2</sup> - 3.5 x 10<sup>2</sup>).

The mean colony counts of different isolates from the various samples sites for the 6 weeks are as follows: Total viable bacteria

(3.2 x 10<sup>3</sup> - 5.4 x 10<sup>3</sup>), total coliform (1.4 x 10<sup>2</sup> - 4.6 x 10<sup>2</sup>), *Escherichia coli* (1.6 x 10<sup>2</sup> - 2.3 x 10<sup>2</sup>), *Staphylococcus aureus* (2.5 x 10<sup>2</sup> - 3.7 x 10<sup>2</sup>), *Shigella* spp. (1.7 x 10<sup>2</sup> - 2.2 x 10<sup>2</sup>), *Klebsiella pneumoniae* (1.1x 10<sup>2</sup> - 1.4 x 10<sup>2</sup>), *Enterobacter aerogenes* (1.5 x 10<sup>2</sup> - 2.8 x 10<sup>2</sup>), *Pseudomonas aeruginosa* (1.1 x 10<sup>2</sup> - 1.4 x 10<sup>2</sup>) and *Saccharomyces cerevisiae* count (2.9 x 10<sup>2</sup> - 3.5 x 10<sup>2</sup>) [Table 2].Thus the Pito drinks analyzed were found to be within the permissible limits of acceptable microbiological quality [29,30]. There was no significant association between the microbial count and the three sampling sites (P-value = 0.919).

#### 4. DISCUSSION

The six different bacteria as well as the fungus isolated from the Pito samples, in this study, were all within acceptable limits of microbial quality. Indigenous foods and drinks such as beverages form part of the culture of human society. While pito seems to serves as refreshment and constitutes a source of economic return for the alcoholic beverage producers in the rural areas, it has not always been possible to have absolute control over the processing of indigenous foods and drinks, particularly in developing countries. This may be due to varied reasons, including inadequate knowledge of food processing and handling practices by processors and vendors. A study which looked at the nutritional composition and microbial analysis of pito, it was found that, in addition to essential mineral elements such as calcium, magnesium and iron, the pito samples also contained microorganisms such as

Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Streptococcus species, Proteus species. stolonifer, Rhizopus Aspergillus flavus. Aspergillus niger, Saccharomyces cerevisiae and Mucor species [1]. The presence of these microorganisms was also attributed to poor handling during production. Similar isolates were found in varying percentage occurrence from the three sampling points in the current study. This emphasizes the close association of these organisms to pito. Even though, the microbes isolated from the samples were found to be within acceptable limits of microbiological quality [29,30], the isolates are potentially pathogenic and therefore if found above the limits, could attract public health attention.

In the present study Saccharomyces cerevisiae was the only fungus isolated and had the highest mean percentage of occurrence (20.8%) than all the bacterial isolates. This observation is not surprising since this fungus is known to be involved in fermentation [14,15,17] and could therefore have been implicated in the fermentation the pito. of Apart from Saccharomyces cerevisiae, the bacterial isolates from the pito samples can be considered microbial contaminants even though the quantities were within the acceptable limits. In a similar study. lactic acid bacteria isolates were found to be involved in the various production stages of Pito [31]. Staphylococcus aureus is a normal flora of the skin, and the common ethological agent of septic arthritis [32]. Its presence in the sample may be attributed to poor handling during production, packaging and selling. Escherichia coli is a very important member of the coliform group. It is part of the normal flora of the intestine of human and

vertebrates. In spite of this, some strains of E. coli can cause gastroenteritis, urinary tract infection [33] as well as diarrhoea in infant [34]. Therefore, identification of this bacterium in the pito samples could merit public health attention. In a related study, which reported the presence of coliforms in hawked Kunun-Zaki drink (A sorghum based but non-alcoholic beverage) widely consumed in Nigeria, the presence of the coliforms was attributed to the use of contaminated water, containers, as well as unhvaienic environment where the drinks were processed and even hawked [35]. Similar factors could account for the presence of the coliforms in the pito samples examined. Pseudomonas aeruginosa which was isolated in all the pito samples in this study is ubiquitous, mostly saprophytic, commonly found in water, soil or other moist environments [36]. It is an opportunistic pathogen and can cause urinary tract infections, respiratory system infections, and gastrointestinal infections, among others [37]. The presence of this organism in the pito samples might be due to the use of ordinary (unsterilized) water in the dilution of the finished pito before serving and drinking. Klebsiella pneumoniae found in the pito samples is widely distributed in nature, occurring both as commensals in the intestines and as saprophytes in soil and water. It has become a very important cause of nosocomial infections [36]. It causes pneumonia, urinary infection, other pyogenic infections, septicemia, meningitis and rarely diarrhoea. Some strains of K. pneumoniae have been shown to produce an enterotoxin [36], therefore pito consumers could be at risk of suffering from health problems associated with the enterotoxin, if such strains were to have been isolated. Enterobacter aerogenes also found in

Isolates *	Location				
	Prampram Keba Shoo	Prampram Alata	Prampram Abia		
Bacteria			-		
E. coli	2.3 x 10 <sup>2</sup>	1.8 x 10 <sup>2</sup>	1.6 x 10 <sup>2</sup>		
S. aureus	3.7 x 10 <sup>2</sup>	3.1 x 10 <sup>2</sup>	2.5 x 10 <sup>2</sup>		
Shigella spp.	2.1 x 10 <sup>2</sup>	2.2 x 10 <sup>2</sup>	1.7 x 10 <sup>2</sup>		
K. pneumoniae	1.1 x 10 <sup>2</sup>	1.4 x 10 <sup>2</sup>	1.9 x 10 <sup>2</sup>		
E. aerogenes	1.9 x 10 <sup>2</sup>	1.5 x 10 <sup>2</sup>	2.8 x 10 <sup>2</sup>		
P. aeruginosa	2.1 x 10 <sup>2</sup>	3.8 x 10 <sup>2</sup>	2.4 x 10 <sup>2</sup>		
Total coliform	1.4 x 10 <sup>2</sup>	3.2 x 10 <sup>2</sup>	$4.2 \times 10^2$		
Total viable bacteria	5.4 x 10 <sup>3</sup>	3.3 x 10 <sup>3</sup>	3.2 x 10 <sup>3</sup>		
Fungus					
S. cerevisiae	3.5 x 10 <sup>2</sup>	2.9 x 10 <sup>2</sup>	$3.2 \times 10^2$		

Table 2. Mean Color	ny Count (CFU/ml	) of isolates in	pito samples	from various	sample sites
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\* According to the compendium of microbiological criteria for food by foodstandards [29], the normal microbial limits for all the bacteria isolates is < 10<sup>4</sup> CFU/ml, with the exception of E. coli which is < 10<sup>2</sup> CFU/ml

this study can cause urinary tract infections and hospital-acquired infections. They are widely distributed in humans and animals, as well as in water, sewage, and soil. Since asymptomatic carriers had been associated with Shigella spp [38], their presence can be attributed to both contaminated water and such asymptomatic carriers, who might be working in the brew house [39,40]. Unhygienic way of serving this local alcoholic beverage, coupled with poor storage, makes the pito drink attract flies that pitch on it and sometimes even fall into it. Therefore, another possible route of transmission could be by insects such as house flies, fruit flies [41] and cockroaches [42] that may reside in and around the brew house.

# 5. CONCLUSION

The current study demonstrated the presence of different bacteria, as well as Saccharomyces cerevisiae in the pito samples. Even though potentially pathogenic microbes were isolated, the colony forming units were within the permissible limits for microbiological quality. However, these microbes, when found in unacceptable quantities in the fermenting samples, could raise public health concern. Therefore, it is recommended that, authorities who regulate food and drink production, maintain their routine education on safety of food and drinks for the pito handlers. This is to further reduce microbial contamination, while periodically screenina locally-produced beverages and their producers.

# **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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