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Investigation of Parasites and Microbial Load in Local Beverages Sold by Vendors in the Port Harcourt Metropolis, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author ECN designed the study and wrote the first draft of the manuscript. Authors OO and OC wrote the protocol, performed the statistical analysis, managed the analyses of the study. Authors OT and PNA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine the prevalence and distribution of gastrointestinal parasites and microbes in Fura da Nono, Gbagaba (Washing and setting), Agbo-iba (Malaria) and Agbo-jedi jedi sold by street vendors in Rivers State.
Study Design: A total of 216 samples were procured randomly from eight different locations.
Place and Duration of Study: Department of Animal and Environmental Biology [parasitology unit), University of Port Harcourt, from March - July 2022.

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Methodology: The 216 samples were examined for gastrointestinal parasites using the Concentration method, while 120 of the samples selected based on type and location were examined for bacteria and fungi using standard microbial techniques.
Results: out of the 216 samples examined, 105(48.61%) local made drinks were contaminated with parasites as follows; Gbagagba (washing & setting) 48(81.3%), Agbo jedi-jedi 24(44.4%), Agbo Iba 22(40.7%) and Fura da Nono 11(20.4%) with the following species of parasites; Hookworm 48(22.2%), *Ascaris lumbricoides* 34(15.7%), and *Trichuris trichiura* 23(10.6%) p=.05. Of the120 local drinks subjected to bacterial evaluation, 92(76.67%) were positive for bacteria as follows; Fura da Nono 26(86.7%), Agbo jedi-jedi 24(80%), Gbagagba 22(73.3%), and Agbo jedi jedi 20(66.7%), with the following species of bacteria; *Staphylococcus aureus* 49(40.83%), *Escherichia coli* 16(13.3%) and *Klebsiella spp* 27(22.5%) p=.05. while 73(60.8%) was positive for fungi as follows; Fura da Nono 22(73.3%), Agbo Jedi-Jedi 21(70%), Gbagagba 12(56.7%) and Ago-Iba 13(43.2%). *Candida specie*s were the only fungi observed in the drinks. This study has shown various microorganisms present in locally made drinks which could have resulted from contaminated soils or poor hygiene during preparation processes.
Conclusion: Street vendors who produce these drinks should be regularly trained on safe handling of their products and send them to approved laboratories for quality assessment before marketing.

Keywords: Gastrointestinal parasites; bacterial evaluation; food safety guidelines; ready-to-eat foods.

1. INTRODUCTION

"In recent times, there has been an upsurge in the consumption of ready-to-eat food and drinks, this is probably due to their convenience. Ready-to-eat foods occur in varieties of food including fruits, fruit juices, nutritional drinks and snacks etc. Generally, they are food items consumed on purchase from vendors, hawkers and consumed immediately without any further preparation. Most Ready-to-eat foods are found in public places including markets, motor parks, and streets, outside schools, hospitals and even expressway" [1]. Among, the various ready-to eat food and drinks sold by street vendors in Nigeria, locally-made drinks represent a higher percentage of consumption as they are consumed by millions of people [2]. "Apart from traditional reasons, these drinks serve many purposes; refreshment, nutritional and medical. The drinks are available and are found in most towns and cities including the city of Port Harcourt. These provide a high percentage of daily energy needs, ranging from 13% to 50% for both children and adults, in developing countries" [3]. "Most of the locally made food and drinks are associated with a common attribute; they are produced outside government regulations without regard to standard food safety guidelines" [4].

"In Nigeria, there are several local unregulated drinks produced by microbial fermentation that are important and tied to the socio-economic lives of many people. A good number of families rely on home-made beverages and sell them in the streets to provide additional source of income. Some drinks, such as fermented palm wine and burukutu, are alcoholic whereas others, such as nunu, are milk-based local yoghurts from animal or plant milk. Other plant-based milk products include tiger nut milk and soymilk. The beverages kunu and zobo may be sugar-sweetened during production with table sugar or other artificial sweeteners with many variants of the drinks sold locally" [2]. Also, some of them are specifically medicinal, such as Agbo (Agbo jedi jedi used for dysentery or to cure temporary high sugar intake and AgboIba for malarial treatment) [5]. Epidemic disease outbreaks from the consumption of contaminated products in Nigeria could occur, as a result of the reluctance of public health authorities in monitoring the production process and consumption of locally made drinks and street foods sold by vendors [6].

"Globally, in the year 2010 a total of 420,000 deaths and 600 million illnesses were attributed to foodborne diseases by infectious agents acquired from consumption of locally made food and drinks" [7]. "The highest population affected was from Africa followed by Southeast Asia, with diarrheal disease agents being implicated as the major contributor, responsible for over 50% of the deaths" [8,7]. "Hence, bacteria and parasites remain the main causative agent relating to foodborne diseases and get into food substances through contaminated soil to plant interaction, water used in processing the drinks, food handlers, and other environmental factors" [9]. "In developing countries, *Vibrio cholera*, *Salmonella typhi*, *Enterotoxigenic Escherichia*

coli, *Campylobacter jejuni*, *Polio*, and *Shigella* species are the prevalent food-borne disease-causing organisms" [10,11]. "Protozoan and helminthic parasites such as *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium species*, *Ascaris lumbricoides*, and *Enterobius vermicularis* are also the main agents of food-borne diseases" [12]. "These organisms, especially in locally made drinks contamination pose a significant threat to food consumers" [10,11]. "The transmission of these infectious agents might occur through fingers, nails, water, and food contaminated with faeces representing the role of person-to-person oral-fecal transmission" [13] "Symptoms of foodborne parasitic infections vary greatly depending on the type of parasite. Protozoa such as *Cryptosporidium* spp., *Giardia* intestinalis, and *Cyclospora cayetanensis* most commonly cause diarrhea and other gastrointestinal symptoms. Helminthic infections can cause abdominal pain, diarrhea, muscle pain, cough, skin lesions, malnutrition, weight loss, neurological and many other symptoms depending on the particular organism and burden of infection, Foodborne parasite has negative effects on human system and can weaken the patient's health, nutrition, intelligence, and performance, for which it causes many economic losses, as it causes loss of carbohydrates and proteins and blood loss (anemia)" [14].

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Port Harcourt metropolis, Rivers State, Nigeria. It lies in the geographical coordinate of 4° 87' 76" N and 7° 02' 83" E. The vegetation type is tropical rainforest with highly fertile land for the cultivation of crops. The major occupations of the indigenous people is animal husbandry, farming, fishing, palm oil processing, petty trading, with a good number of them in public service, while others own private business enterprises such as fast foods, restaurants, hotels and bars, where local drinks of various kind are consumed. Also, Port Harcourt metropolis most of these local drinks are sold by street vendors at various spot and hawked around its environment

2.2 Sample Collection

A total of 216 samples of local made drinks were bought from local street vendors in plastic bottles and white nylon from 8 different locations around Obio/Akpor and Port Harcourt metropolis from March-July 2022. Twenty-seven samples of these drinks were gotten from each location; Rumuola, Nkpolu, Rumuigbo, Choba, Trailer Park Onne, Gambia market mile 1, Yam zone Creek Road and Cattle market Oyigbo. These areas are known to be commercial and industrial with a wide ethnic diversity in each location. Most of the areas are populated by the Yorubas who take more of the herbal concoctions and the Fulanis who take more of the Fura da Nono. Out of a total of 216 samples, 120 were subjected to bacterial and fungal test due to the cost of materials.

2.3 Examination of Samples for Bacteria

2.3.1 Preparation of culture media

The media used were prepared according to the manufacturer's instructions. They were Nutrient Agar (NA), *Salmonella Shigella* Agar (SSA), Sabouraud Dextrose Agar (SDA) and MacConkey Agar (MA).

2.4 Characterization of Isolates

2.4.1 Gram staining of the isolates

A smear was prepared from a 24-hour fresh culture and heat-fixed by passing over a flame and subsequently, the smear was flooded with crystal violet for 1 minute and rinsed under the slow flowing tap, again, the smear was flooded with gram's iodine for 1 minute and rinsed, it was flooded with ethanol for 20 seconds and rinsed, then finally safranin was added for about 1 minute and washed off with water. The slides were allowed to air dry and viewed under the light microscope using oil immersion objective lens, cells that appeared purple were recorded as gram-positive cells while cells that appeared pink under the microscope were recorded as gram-negative cells [15].

2.5 Biochemical Tests for Identification of Bacteria

The biochemical test involved hydrogen peroxide test, oxidase test, citrate slant and urease test.

2.5.1 Oxidase test

A piece of filter paper was soaked in 1% solution of oxidase reagent (tetramethyl-p-phenylenediamine- dihydrochloride) prepared by standard procedure. Sample of growth from the

nutrient agar slant was obtained using a sterilized platinum wire loop and smeared on the moistened piece of paper. The development of an intense purple color by the cells within 30 seconds indicates a positive oxidase test.

2.5.2 Hydrogen peroxide test

The test demonstrates the presence of catalase which is an enzyme that catalyzes the release of oxygen from hydrogen peroxide (H_2O_2). A colony of 24 hours old culture was picked using a sterile loop and then emulsified in a few drops of hydrogen peroxide on a clean slide. The presence of effervescence indicated catalase positive reaction whereas negative reaction showed no effervescence.

2.5.3 Citrate utilization test

The Citrate test uses a medium in which sodium citrate is the only source of carbon and energy. If an organism can use citrate as the sole source of carbon and energy, it will need to use ammonium salts for nitrogen. This will result in the release of ammonia, causing a color change in the medium from green to blue. Tubes of Simon's citrate agar were each inoculated with a test organism and incubated at 35^0C for 48 hours. A change in the medium from green to royal blue was recorded as a positive test [16].

2.5.4 Urease test

Isolates were inoculated into liquid urea agar supplemented with urea and aseptically dispensed into sterile bijou bottles and slanted to solidify. They were incubated at 37^0C for 24-48 hours. The development of bright pink or red color indicates positive urea reaction.

2.5.5 Indole test

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole, which accumulates in the medium. The isolated colony of the test organism was emulsified in tryptophan broth (peptone water) and incubated at 37°C for 24 hours in ambient air. 0.5 ml of Kovac's reagent was added to the broth culture down the side of the tube and observed for color change at meniscus. The development of a -red color (benzaldehyde reagents) within 20 seconds indicates the presence of indole. A negative test is colorless or slightly yellow.

2.6 Fungal Identification

The fungal isolates were identified by their distinct appearance using a Sabouraud dextrose agar. They were also identified microscopically by placing a drop of the sample Aliquot on a clean grease free slide and covered with a cover slip and viewed at 40x magnification.

2.6.1 Examination of samples for parasites

Aliquots of the pooled saline washes were examined microscopically for the presence of parasites as follows;

2.6.2 Direct wet preparation method

Using a plastic pipette, normal saline was placed at one end of a clean grease-free glass slide and iodine was placed on the other end and a drop of the sample Aliquot was emulsified at both ends and carefully covered with coverslips to prevent formation of bubbles. The smear was examined using a low power (10x) objective and then again by using a high power (40x) objective lens respectively. The eggs/ova, cyst and larvae of parasites were identified with reference to Atlas of Parasitology [17].

2.6.3 Sedimentation technique

Five (5 ml) of 10% formalin was transferred into a centrifuge tube followed by 2ml of the sample Aliquot and 3ml of ether was added raising it to the 10 ml mark. A stopper was placed at the top of each tube and shaken. The tubes were then centrifuged for 5 minutes at a speed of 2500rpm. After centrifugation, the supernatant was discarded by gently inverting the tubes leaving the deposits in the tube and the sediments were then re-suspended. A drop of the deposits was placed on a clean slide and covered with a cover slip for examination under a microscope at 10x and 40x objective lenses respectively. Parasites were identified by the morphological structures of their cysts, ova or larvae using Atlas of Parasitology [17].

2.7 Statistical Analysis

Data collected were analyzed using Microsoft excel 2016 version. The ANOVA analysis was used to determine the association between contamination (of parasite and bacteria) and type of samples, and the association between contamination (of parasite and bacteria) and location, while the T-test analysis was used to determine the association between fungal contamination and type of sample, and the association between fungal contamination and location. P values less than or equal to 0.05 were considered significant.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Bacterial analysis

A total of 120 samples were subjected to bacterial analysis, 92(76.67%) were positive for the following bacterial isolates; *Staphylococcus aureus* 49(40.83%), *Klebsiella* spp 27(22.5%) and *E. coli* 16(13.3) Respectively.

The locally made drinks were positive for bacteria as follows; Fura da Nono 26(86.7%), Agbo jedi-jedi 24(80%), Gbagagba 22(73.3%), and Agbo jedi jedi 20(66.7%) Respectively. The 8 locations for the study were positive for bacteria as follows; Choba 13(86.7%), Oyigbo 13 (86.7%), Mile 1 12(80.0%), Rumuola 12(80.0%), Nkpolu 11(73.3%), Creek Road 11(73.3%), Onne 10(66.7%) and Rumuigbo 10(66.7%) Respectively (Table 1).

3.1.2 Fungal Analysis

A total of 120 samples were subjected to fungal analysis, 73(60.8%) were positive for *Candida* species as follows; Fura da Nono 22(73.3%), Agbo Jedi-Jedi 21(70%), Gbagagba 12(56.7%) and Ago-Iba 13(43.2%) Respectively.

Out of the 8 locations, 5 locations were positive for fungi as follows; Choba 7(47.7%), Creek Road 5(33.3%), Rumuola 4(26.7%), Nkpolu 2(13.3%), and Mile one 1(6.7%) respectively (Table 2).

3.1.3 Parasitological analysis

A total of 216 locally made drinks were examined for gastrointestinal parasites, 105(48.61%) were contaminated with parasites as follows; Gbagagba (washing and setting) 48(81.3%), Agbo jedi-jedi 24(44.4%), Agbo Iba 22(40.7%) and Fura da Nono 11(20.4%) with the following parasites; Hookworm 48(22.2%) *Ascaris lumbricoides* 34(15.7%), and *Trichuris trichiura* 23(10.6%).

Samples from the 8 locations were positive for parasites as follows; Cattle market Oyigbo 23(62.9%), Gambia market Mile one 16(59.3%), Trailer Park Onne 15(55.56%), Yam zone Creek Road 14(51.85), Nkpolu 12(44.44%), Rumuola 10(37.04%), Rumuigbo 9 (33.33%) and Choba 6(22.22%) (Table 3).

Table 1. Bacteriological analysis results

Bacteria	No. Examined	No. Infected (%)	*E. coli*	*Klebsiella spp*	*S.aureus*
Local Drinks					
Gbagagba (washing & setting)	30	22(73.3)	0	14(46.7%)	8(26.7%)
Agbo-Iba (Malaria)	30	20(66.7)	0	8(26.7%)	12(40%)
Agbo Jedi-Jedi (Dysentry)	30	24(80)	16(53.3%)	5(16.7%)	3(10%)
Fura da Nono	30	26(86.7)	0	0	26(86.7%)
Total	**120**	**92 (76.7%)**	**16 (13.3)**	**27 (22.5)**	**49(40.83)**
	SD=7.8	**df=14**	**F=0.89**		**P=0.05**
Location					
Rumuola	15	12 (80.0)	10 (66.7)	2 (13.3)	0
Nkpolu	15	11 (73.3)	0	6 (40.0)	5 (33.3)
Rumuigbo	15	10 (66.7)	7 (46.7)	3 (20.0)	0
Choba	15	13 (86.7)	11 (73.3)	0	2 (13.3)
Onne	15	10 (66.7)	2 (13.3)	8 (53.3)	0
Mile one	15	12 (80.0)	9 (60.0)	3 (20.0)	0
Creek road	15	11 (73.3)	3 (20.0)	2 (13.3)	6 (40.0)
Oyigbo	15	13 (86.7)	6 (40)	0	7 (46.7)
Total	**120**	**92 (76.7)**	**48 (40)**	**24 (20)**	**16 (16.7)**
	S. D=3.53	**df=23**	**F=2.62**		**P=0.05**

E. coli = Escherichia coli., S. aureus=Staphylococcus aureus

Table 2. Fugal analysis result

Fungi	No. Examined	No. Infected (%)	*Candida spp*
Drink Type			
Gbagagba	30	17(56.7)	17(56.7)
Agbo-iba	30	13(43.2)	13(43.2)
Agbo jedi-jedi	30	21(70)	21(70)
Fura da Nono	30	22(73.3)	22(73.3)
Total	**120**	**73(60.8)**	**73(60.8)**
S. D= 4.11	**Df=3**	**t.stat=1.823**	**P=0.05**
Location			
Rumuola	15	4 (26.7)	4 (26.7)
Nkpolu	15	2(13.3)	2 (13.3)
Rumuigbo	15	0	0
Choba	15	7(46.7)	7 (46.7)
Onne	15	0	0
Mile one	15	1(6.7)	1(6.7)
Creek road	15	5(33.3)	5 (33.3)
Oyigbo	15	0	0
Total	**135**	**19 (14.1)**	**19 (14.1)**
SD=2.67	**df=7**	**t.stat=0.31**	**P=0.05**

Table 3. Parasitological analysis results

		Prevalence (%)			
Local Drink	**Number of examined**	**Number infected (%)**	***Hookwom***	***Ascaris lumbricoides***	***Trichuris trichiura***
Gbagagba (washing & setting)	54	48 (81.3)	20(37.0)	14(25.9)	14(25.9)
Agbo-Iba (Malaria)	54	22 (40.7)	15(27.8)	5(9.3)	2(3.7)
Agbo Jedi-Jedi (Dysentry)	54	24 (44.4)	10(18.5)	7(12.9)	7(12.9)
Fura da nono	54	11(20.4)	3(5.9)	8(14.8)	0
Total	**216**	**105(48.6)**	**48(22.2)**	**34(15.7)**	**23(10.6)**
	SD=3.75	**df=14**	**F=1.55499**	**P=0.251**	**P=0.05**
Locations					
Rumuola	27	10 (37.0)	5(18.5)	3(11.1)	2(7.4)
Nkpolu	27	12(44.4)	4(14.8)	2(7.4)	6(22.2)
Rumuigbo	27	9 (33.3)	5(22.7)	2(7.4)	2(7.4)
Choba Market	27	6 (22.2)	3(11.1)	2(7.4)	1(3.7)
Trailer Park, Onne	27	15(55.6)	7(25.9)	6(22.2)	2(7.4)
Gambia market (Mile 1)	27	16(59.3)	7(25.9)	5(18.5)	4(14.8)
Yam zone, Creek Road	27	14 (51.8)	4(14.8)	7(25.9)	3(11.1)
Cattle market, Oyigbo	27	23(62.9)	11(40.47)	7(25.5)	5(18.5)
Total	**216**	**105(48.6)**	**46(21.2)**	**34(15.7)**	**25(11.5)**
	SD=2.32	**df=23**	**F=2.70**	**P=0.090**	**P=0.05**

3.2 Discussion

Herbal medicine in form of drinks are commonly used by people for the treatment of some ailments. Fura da Nono is a common drink among the Fulani's and it is known for its great importance as it aids digestion and prevents constipation and also represents part of their cultural heritage. In this study, the overall prevalence of parasite contamination of the Local drinks was (48.6%), thus providing valuable information on the burden of intestinal parasites in local drinks commonly consumed in Rivers state, Nigeria. This study revealed that

Hookworm eggs was the most prevalent parasite accounting for (22.2%) which is not in line with the study of Onyemelukwe et al. [18] where *Ascaris lumbricoides* was observed as the most prevalent in the local made drink**.** This could be as a result of contaminated water or poor handling of the plant parts used in production process of these drinks. *Hookworm* is found in the faeces and man get infected when he comes in contact bare footed on contaminated soil [19]. Gbagagba (Washing and setting) which is mostly taken by the Yoruba tribe of Nigeria for the treatment of ailments such as gonorrhea, candidiasis, waist and back pain, chronic typhoid fever and optimization of blood sugar level had a high parasite contamination (81.3%). In Cattle market Oyigbo and Gambia market (Mile 1) Port Harcourt where (62.9%) and (59.3%) parasite prevalence was recorded; it was observed that poor water channels and lack of portable water or poor hygiene level of the producers might have contributed the results. This is in line with the work of Posadzki et al. [20] who implicated parasites as contaminants and adulterants of herbal medicinal products. Thus, it is likely that parasitic contamination of local made drinks found in this research may have come from contaminated water or herbs, food handlers and as well utensils used in the production of the drinks.

Local made drinks subjected for bacteriological evaluation were contaminated with three species; *Escherichia coli*, *Staphylocoocus aureus*, and *Klebsiella spp.*, which is in line with the study of Idu et al. [21]; Oluyege and Adelabu [22] who also in their studies isolated *Staphylococcus aureus*, *E. coli.*, *Salmonella typhi, Pseudomonas aeruginosa, Serratia marcescens, Klebsiella pneumonia and Proteus mirabilis*.

Most *Klebsiella* of medical importance account for a significant proportion of urinary tract infections and soft tissue infections treated in hospitals [23]. *Escherichia coli* which was also isolated in this study remains one of the most frequent causes of several common bacterial infections in humans as it is the prominent cause of urinary tract infection, septicemia and other clinical infections such as neonatal meningitis [24]. Staphylococcus aureus which was isolated in this study is a gram-positive bacterium that causes a wide variety of clinical diseases on the skin, however if allowed into the internal tissues or bloodstream, these bacteria may cause a variety of infections [25]. Herbal medicines harbour various types of microorganisms because the trees and plants from which they are made have microorganisms adhered to their stems, barks, leaves, flowers, fruits and roots. Also, the microbial contamination of herbal medicines depends on several environmental factors, including the gross pollution in our environment, coupled with the sub-standard processing methods of the concoctions. Microbial contamination may also come from dust, atmosphere and contaminated utensils, water and those preparing them. This therefore encourages an almost similar distribution of these organisms, both qualitatively and quantitatively which differences were not statistically significant (p=0.5) among the batches of tested herbal medicines.

Many of these bacteria are enteric suggesting inadequate hygienic practices on the side of producers and marketers suggesting the access of unwholesome materials like faecal material/wastes into these herbal medicines. Also suggests that access to the herbs might have been by way of a polluted environment, contaminated water used in the preparations, contaminated utensils, and contaminated herbs/plants as well as directly from handlers. *Klebsiella sp*. ranked highest and this may be as a result of inadequate heat processing, improper handling of products and contaminated processing equipment probably through the soil [26].

Also (60.8%) of the Local drinks subjected for fungal evaluation were contaminated with only Candida *spp* which is not in line with the study of Taylor and Unakal [25]; Frazier and Westhoff [26] who isolated (*Basidiobotrytis spp*, *Oedocephalum spp*, *varicosporium spp* and *Articulospora inflata*). From this study, *Candida spp* was prevalent in this local made drink (Fura da Nono, Washing and setting, Agbo-iba, Agbo jedi-jedi). Candida can cause infections if it grows out of control or if it enters deep into the body. For example, it can cause infections in the bloodstream or internal organs like the kidney, heart, or brain [26].

4. CONCLUSION

This present study has shown that there are varieties of microorganism present in our various local made drink which could have resulted from contaminated soils, plants and its products, preparation processes, quality of water, containers and processing equipment. There was a moderately high contamination of parasites and

bacteria of both economic and zoonotic importance in the locally made drinks in Rivers State. This could be a source of many enteric and other infections that abound, and stress the pressing need for standardization and regular tests of these locally made drinks by appropriate agencies. and thus, minimize the negative impacts on public health.

5. RECOMMENDATION

i. Proper hygienic conditions should be maintained in all preparation processes starting from collection, processing, packaging and storage.
ii. There is need for mass education to enlighten the public on excessive consumption of locally made drinks since many microorganisms were isolated from this study.
iii. Street vendors who engage in locally made drinks should be encouraged to send their products regularly to laboratories for quality assessment to ensure consistency and quality before marketing.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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