Bacteriological Quality And Public Health Implications Of Fermented Cassava (Garri) Sold In Okwor And Nkalagu Markets In Ebonyi State, Nigeria.

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Abstract: Food security has been a major challenge to the world populace over the last few centuries because of the alarming concern of disease outbreak caused by consumption of contaminated food and food products. This study determined the bacteriological quality of fermented cassava (garri) sold in Okwor and Nkalagu markets in Ebonyi State, Nigeria. A total of sixteen (16) samples (8 white and 8 yellow) were purchased from the two markets and processed using standard procedures. The results revealed a high microbial burden in the garri samples examined ranging from 6.6 x 10^6 to 1.07 x 10^7 Aerobic Plate Counts (APC). The pH values of the garri samples purchased from both markets ranged from 5.47 to 6.61. Out of the sixteen samples, a total of 32 bacterial isolates were obtained, out of which 14(43.8%) were *Staphylococcus aureus*, 6(18.8%) *Escherichia coli*, 5(15.6%) *Bacillus cereus*, 4(12.5%) *Pseudomonas aeruginosa*, 2(6.2%) *Streptococcus* species, while the least occurring isolate was *Yersinia* species with recovery rate of 3.1%. *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus* species were isolated from Okwor market while *Yersinia* species, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus* were isolated from Nkalagu market. The study reveals unacceptable bioload in garri from both markets. The heavy bacterial contamination and vast array of bacteria isolated from the garri sold in both markets portend alarming danger posed by consumption of garri sold in these markets. Therefore renewed vigilance on the efficacies of food processing conditions, handling techniques and handlers technical knowhow, personal hygiene practices and safety of finished products are hereby recommended.

Index Terms: Bacteria, Cassava, Garri, pH, Nigeria.

1 INTRODUCTION

Food security has been a growing concern worldwide, due to increase in the world population. This has led to an unprecedented global interest in agriculture. For instance, in 2011, the administration of President Goodluck Ebele Jonathan of Nigeria launched an Agricultural Transformation Agenda to promote agriculture as a business, integrate the agricultural value chain and make agriculture the key driver of Nigeria's economic growth [1]. Today, cassava (Manihot esculenta Crantz) is the chief source of dietary food energy for the majority of the people living in the low land tropics, and much of the sub-humid tropics of West and Central Africa [2]. It supplies about 70% of the daily calorie of over 50 million people [3] in Nigeria and about 500 million people in the world [4]. It is processed into garri, lafun, tapioca and kokote. Garri is an important by-product of cassava being an important item in the menu of most Nigerians. It is particularly popular because of its ready-to-eat nature [4]. Garri is a good source of energy and fibre. Other nutrients are also present in marginally nutritional significance [5]. The production of garri is a burdensome and tasking procedure and its method of production differs from one locality to another, garri is typically produced by peeling the cassava tubers, washing and grating them, which is packed into closely woven bags [6],[7]. After fermentation, frying at high temperature dries the fermented pulp to about 10% moisture content and this may result in partial dextrinization of starch [8],[9]. Also, high temperature destroys both enzymes and microbes present in the fermented garri, as well as eliminates cyanide gas from the garri product. The post processing problems associated with garri include loss of microbial stability and spoilage during storage, distribution and marketing. The sale of garri in the local markets in Nigeria is associated with practices such as open display in bowls, open buckets and mats at points of sale and the use of bare hands in handling and selling of garri products. These unhygienic practices, may lead to microbial contamination due to deposition of bioaerosols on exposed products, transfer of microbes from dirty hands and utensils [10]. Frequent visits by animals and fomites (which may carry infectious agents), can contribute to the post-processing problems of this product. The

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variety of microbial populations that come in contact with a particular food material depends on the nutritional status, pH, water content as well as the nature of the organism [11]. These microorganisms can cause deterioration in food quality and spoilage, serious food borne illnesses and may pose a threat to public health. Moreover, the source of these microbial contaminants may also be a portal for contamination by pathogenic microbes [12]. The nature of garri and its readiness for eating has made it a common practice in Nigeria especially among students to eat garri raw or as snacks, without considering the bacteriological implications [11]. Hence, this study evaluated the bacteriological quality of garri sold in Okwor and Nkalagu Markets in Ebonyi State, Nigeria with a view to ascertaining the public health implications.

2 MATERIALS AND METHODS

2.1 Study Area

The study was carried in two markets (Okwor and Nkalagu) located at Ohaukwu and Ishielu local Government areas respectively, both in Ebonyi State, Nigeria. Ohaukwu is a Local Government Area of Ebonyi State, Nigeria. It is an agrarian area of 517 km² and a population of 196,337. The area is bounded by longitudes 7°55'-8°00 and latitude 6°20'IM-6°25'. The map area is located at the north-western part of Abakaliki town. Ishielu is a Local Government Area of Ebonyi State, Nigeria with headquarters at Ezillo. It is an agrarian area of 872 km² with a population of 151,048 at Latitude and Longitude of Ishielu is 5.1216 and 7.3733 respectively.

2.2 Collection of Samples

The study was carried out between March and April, 2016. A total number of sixteen (16) samples were purchased from two major markets in Ohaukwu (Okwor market) and Ishielu (Nkalagu market) Local Government areas. Four (4) each of yellow and white garri types were randomly purchased from each market from four different but major garri food vendors per market. The samples were labelled appropriately to indicate the name of the market, garri type (yellow or white), sample number, date and time of collection. Samples were transported in sterile bowls to the laboratory for analysis within 24 hours of collection.

2.3 Determination of pH

The pH of the samples was determined following the method described by Ogiehor and Ikenenomeh [13]. In this, 10 g of each sample were homogenized in 10 ml of distilled water and the pH of the suspension determined using a reference glass electrode pH meter (Mettler Delta 340, Mettle- Tocedo Limited, UK).

2.4 Determination of moisture content

Two grams of each of the sample was weighed into dried weighed crucible. The samples was put into a moisture extraction oven at 105 ^{0}C and heated for 3 hours. The dried samples was put into desiccators, allowed to cool and reweighed. The process was reported until constant weight was obtained. The difference in weight was calculated as a percentage of the original sample Percentage moisture.

$$\text{Percentage (\%) Moisture Content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

W_1= Weight of empty petri dish

W_2= Weight of petri dish + Sample before

W_3= Weight of empty petri dish + Sample after

2.5 Microbiological Analysis

Ten gram (10g) of each sample of garri were homogenized in 9ml of sterile distilled water (10^{-1} dilution), further serial dilution of sample homogenate to 10^{-4} was carried out also in a sterile distilled water, transferring 1ml of initial suspension into subsequent tubes used for the serial dilution. Approximately, a 0.1ml aliquot of appropriate dilution (10^{-4}) was spread on plates of MacConkey agar for coliform count. All culture plates were incubated at 37°C aerobic for 24hrs. Tubes showing gas production and/or colour change of dye were reported as presumptive coliform test positive. These positive tubes were streaked out on duplicate plates of Eosin methylene blue (EMB) agar for confirmatory test. Plates were incubated for 24hrs at 37°C and 44°C respectively. Growth of characteristic colonies on EMB constitute confirmatory test positive. Colonies from confirmatory test were Gram stained and inoculated into lactose broth for completed coliform test. Gas production and/or colour change of dye plus Gram negative non spore bearing rod represent presence of coliform [14]. Absence of growth was recorded at 44°C incubation for all the samples indicating absence of fecal coliform.

2.6 Inoculation of plates

Two different media (Nutrient and MacConkey Agar) were inoculated with approximately 0.1ml aliquots of appropriate dilution (10^{-4}) of each of the samples and incubated at 37 °C for 24 hours. After incubation, the plates were examined for growth and the morphological characteristics of the isolates were recorded.

2.7 Enumeration and Identification of isolates

Colony counts at the end of incubation time were carried out with colony counting chamber and the total microbial population was expressed as colony forming units per gram ($cfug^{-1}$) of sample. The colonies of the pure cultures grown on the solid media were examined with special reference to their sizes, colours, consistency, shape and textures after 24-hour incubation at 37°C. After morphological identification, the isolates were further subjected to Gram reaction and then various biochemical tests including catalase, coagulase, oxidase and indole tests for further identification.

3 RESULTS

The Total Aerobic Plate Count (TAPC) of yellow garri samples collected from Nkalagu revealed the highest count of $1.07x10^7$ with a moisture content of 11.9 and having the pH value of 5.62. This was followed by $9.2x10^6$ with moisture content of 15.1 having pH value of 5.57. The lowest TAPC is $6.6x10^6$ with a moisture content of 15.7, having a pH value of 6.64. The number of coliforms found were Too Numerous To Count (TNTC), in samples 1, 2 and 3 with moisture content of 15.7, 15.7 and 11.9 each having a pH values of 6.64, 5.57 and 5.62 respectively, the lowest coliform count was got from sample 4 with prevalence of 8.8×10^6cfu/ml with the moisture content of 15.5 having a pH value of 6.43. Two samples show too numerous to count in TAPC both having a moisture content of 12.7 and 11.85 having a pH value of 6.07 and 5.58 respectively. Sample 4 shows the highest coliform count with $4.9x10^6$ having the moisture content of 11.85. Out of the four (4) samples analysed, sample 4 showed TNTC with the

moisture content of 15.95 having the pH value of 5.98. The coliform count is 1.5 x 10^6 which is the lowest amongst the four samples. Other samples (1,2,3) show the prevalence of 6.6x10^6, 9.2x10^6, and 1.0x10^6 respectively with moisture content of 12.45, 12.45 and 15.05 respectively, their pH value read 5.93, 5.77 and 5.64 respectively. Details of the results can be seen in Table 1 below:

Table1. *Bacterial Load (cfug^{-1}), Moisture Content, and pH of Garri Samples from Nkalagu and Okwor Markets in Ebonyi State, Nigeria.*

Sample outlet		Yellow Garri				White Garri			
		TAPC	Coliform Count	Moisture content	pH	TAPC	Coliform Count	Moisture Content	Ph
NKALAGU	1	6.6x10^6	TNTC	15.7	6.64	6.6 x 10^6	TNTC	12.45	5.93
	2	9.2 x 10^6	TNTC	15.1	5.57	9.2 x 10^6	TNTC	12.45	5.77
	3	1.07 x 10^7	TNTC	11.9	5.62	1.0 x 10^6	TNTC	15.05	5.64
	4	8.3 x 10^6	8.8 X 10^6	15.5	6.43	TNTC	1.5 X 10^6	15.95	5.98
OKWOR	1	7.2 x 10^6	2.0 X 10^5	14.05	5.80	NG	NG	12.1	6.04
	2	TNTC	1.6 X 10^6	12.7	6.07	TNTC	8.0 X 10^5	12.1	6.32
	3	3.0 x 10^6	8.0 X 10^5	13.05	5.54	4.0 X 10^5	2.0 X 10^6	12.8	5.47
	4	TNTC	4.9 X 10^6	11.85	5.58	TNTC	2.0 X 10^5	13.75	5.58

Key: *TAPC: Total Aerobic Plate Count, TNTC: Too Numerous To Count, NG: No Growth*

The analysis of the garri samples collected from Okwor market revealed the presence of *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus* spp., while those collected from Nkalagu market revealed the presence of *Yersinia* spp., *Pseudomonas aeruginosa*, *Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus*. Details of the results showing the morphological and biochemical characteristics of the isolated organisms and their distribution based on the market and type of garri can be seen in Table 2. In general, as seen in Figure 1, *Staphylococcus aureus* were the most isolated bacterium (43.8%) from both markets, followed by E. coli (18.8%), *Bacillus cereus* (15.6%), *Pseudomonas aeruginosa* (12.5%) and *Streptococcus* spp. (6.2%). *Yersinia* spp. showed the least occurrence with only one isolate with 3.1% frequency.

Table 2: *Morphological and Biochemical Characteristics of the Bacterial Isolates from Yellow and White Garri Samples Purchased from Okwor and Nkalagu Markets in Ebonyi State, Nigeria.*

Market	Morphological and Biochemical Characteristics of the Bacterial Isolates						Probable Organism	
	Morphology	Biochemical Tests					Garri Type	
		Gr	Ox	Ca	Ind	Coa	Yellow	White
NKALAGU	Circular, raised, shinny, smooth, colourless and non pigmented	-	-	+	+	-	-	*Escherichia coli*
	Oval shaped, clusters, creamy, raised, small colony with smooth surface	+	+	+	-	+	*Staphylococcus aureus*	*Staphylococcus aureus*
	Rod shaped, blue-green pigment, small and mucoid colonies.	-	+	+	-	-	*Pseudomonas aeruginosa*	-
	Coccobacillus, small, pink and shiny colonies.	-	-	+	-	+	*Yersinia* spp.	-
	Rod shaped, dry, flat, and irregular.	+	+	+	-	-	*Bacillus cereus*	*Bacillus cereus*
OKWOR	Oval shaped in chains, colourless, dry, shiny or mucoid.	+	-	+	+	-	-	*Streptococcus* spp.
	Oval shaped, clusters, creamy, raised, small colony with smooth surface	+	+	+	-	+	*Staphylococcus aureus*	*Staphylococcus aureus*
	Circular, raised, shinny, smooth, colourless and non pigmented	-	-	+	+	-	-	*Escherichia coli*

Key*: Gr= Gram reaction, Ox= Oxidase test, Ca= catalase test, Ind= Indole test, Coa= Coagulase test,+ = Positive, = Negative*

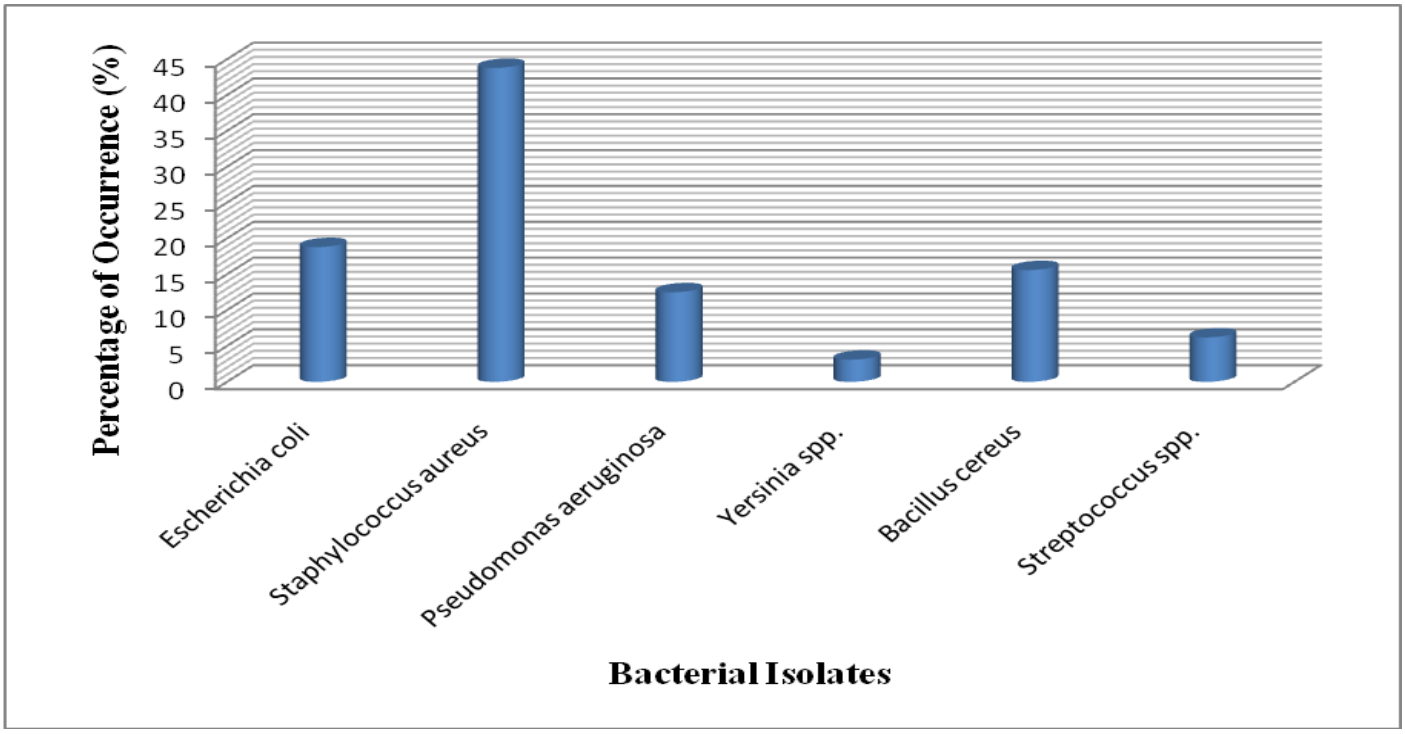

Figure 1: *Frequency of Occurrence of Bacterial Isolates from Yellow And White Garri Samples Examined.*

4 DISCUSSION

In addition to the alarming rate of death and ill health caused by food poisoning, individuals, families, health care system and society as well as commercial enterprises incur tremendous economic loss. These losses include the cost of medical care, the cost of investigating food contamination outbreaks, legal costs and fine [15]. An increasing intake of dry garri as snacks or with cold water is an added practice that exposes the populace to health risk due to the microbial status of garri product. The microbial content of food and food products depicts interplay between pH and moisture contents. The results of this present study showed that the garri samples studied had high pH and moisture content. This study corroborates the work of Ogiehor *et al.* [16] that high pH and moisture content supports the growth of microorganisms in the garri samples. Here, the higher the pH value and moisture content the more the growth of bacteria. This supports that acid is an antimicrobial agent. The high bacteria in this study which is above the acceptable range is not in line with the work of Olapade *et al.* [17] who obtained an acceptable range of plate counts. This can be attributed to differences in pH values. Olapade *et al.* [17] in their study on microbiological quality of fermented cassava (Garri) sold in Ota Ogun State Nigeria showed that the pH values of samples examined ranged from 4.76 to 4.94 in both white and yellow types of garri. While in this present study the pH values of both white and yellow types of garri range from 5.47 to 6.64 which suggest that the unacceptable level of contamination of the garri samples is because of its high pH values. This can be as a result of proper fermentation of garri. The longer it stays the better it removes those unwanted chemicals such as cyanide which is acidic in nature and harmful to human health. In other words, the acceptable counts obtained from the other study can be attributed to the inability of microorganisms to thrive in low pH, low moisture and prevailing conditions in the garri. Organic acids (such as lactate, propionate and acetate) produced during fermentation which lower pH of product may be inhibitory to bacteria [12]. Most times for better availability and sells, producers of garri instead of allowing it for at least four (4) days to ferment conclude the process at day one or two [19]. Therefore, the difference between both studies might not only be due to difference in study area but could also include the processes involved in garri production, considering the dirty environment found also in Ota, Ogun State, Nigeria [17]. Also, the study showed that garri from both markets was heavily contaminated as most of the samples had Average Aerobic Plate Count (AAPC) within unacceptable range i.e. counts ≥ 10^6 [18]. This result agrees with Olapade *et al.* [17] who observed high presence of coliform in their study. The high presence of coliform which is an indication of faecal contamination could be attributed to lack of personal hygiene of handlers as well as the poor sanitary condition of the processing/display environments. It was observed that in both markets, traders of garri were non literate and lack personal hygiene. The market environment which has refuse dumps around it could be the major source of environmental contamination by bioaerosols [16]. The markets lack proper toilet facilities leading to indiscriminate defecation by traders and buyers in the surrounding bushes, further explaining reason for the high faecal contamination recorded in this study; since the study was carried out during dry season, wind and breeze could have easily carried faecal materials from those bushes as aerosols and deposit them on the displayed garri produce. Buyers attitude towards the displayed garri could have contributed to the high bacterial load of the garri as they touch the garri with bare hands to test before they buy. The coliform count of both yellow and white garri types from both markets shows that samples from Nkalagu Market were more contaminated than those from Okwor Market. This shows faecal contamination which can be as a result of defecation around market areas or contamination during garri

processing may be through water or cross contamination by sellers and buyers [19]. Also, it was observed that Fulani herdsmen graze their cattle around the markets environment suggesting a possibility of animal droppings being carried by wind and dust and deposited on the garri product. This is highly possible because the market (Nkalagu) is sited along the major road and also the sample collection was done during dry season. It was also observed that there was more microbial contamination in White Garri than Yellow Garri, which may be due to the antimicrobial property of the oil added to the yellow garri. This is in consonance with the work of Orji *et al.* [20], but contrary to the work of Thoha *et al.* [21], who reported more microbial contamination in Yellow Garri than in White Garri. The variation in these results can be explained by point of sale contamination that could have raised the microbial burden of the Yellow Garri in the study of Thoha *et al.* [21]. More so, the study revealed that garri samples (Yellow and white) collected from both markets (Okwor and Nkalagu) harbour vast array of bacterial isolates which include: *Yersinia* spp, *Pseudomonas aeruginosa*, *Bacillus cereus*, E. coli, *Staphylococcus aureus* and *Streptococcus* spp. The diverse bacterial population contaminating the product could be due to the fact that fermentation of garri is by mixed microbial cultures. Also post process contamination specifically associated with sieving of the products after heat treatment and the spreading of products in the open to air dry, together with the practice of leaving garri open for sale, and the regular unhygienic practice by buyers who using their bare hands fetch garri to taste before buying. The isolation of diverse bacterial species from ready to eat food (garri) is in line with the findings of Mensah *et al.* [22], Idowu [23], Taulo *et al.* [24], Oranusi and Braide [25] and Olopade *et al.* [17]. The major source of contamination in these markets is likely to be from the sellers and the aerosols. This supports the observation of Manlee *et al.* [26] who states that the vendors can be carriers of pathogens like E. coli, S. aureus etc. Some of the microorganisms which are airborne originate from different sources such as soil, animals and humans [27],[28]. Activities such as sewage treatment plants, animals rearing, fermentation, construction works and agricultural activities play a major role in emitting microorganisms into the air [29],[30]. These organisms play a role as an effective infectious aerosol because the organic materials of garri provide essential nutrients for airborne microorganisms that adhered to their surface [31]. The isolation of E. coli from both markets is in line with the results obtained by Lues *et al.* [32], who reported that since the isolation of E.coli and Salmonella which usually resides in animal intestines from garri may be attributed to the indiscriminate dumping of sewage and refuse in the market environment. These could have also resulted from faecal particulates spreading by the flapping of wings during slaughter of birds at chicken processing centres in the markets. Rupturing of the lower intestinal tract during processing of slaughtered animals at the markets at the abattoirs may also be implicated. The absence of Salmonella in this study is in line with the work of Olopade *et al.* [17] on microbial quality of fermented cassava (Garri) sold in Ota Ogun State Nigeria. The bacteria species, *Bacillus cereus*, *Staphylococcus aureus*, E. coli and *Pseudomonas aeruginosa* isolated in this study is in line with Olopade *et al.* [17] who isolated same microorganisms. The consumption of contaminated garri could therefore portend a potential risk to consumers especially with this high microbial load. Also, Bacillus sp., being a spore former and known to withstand environmental stress, may have survived the harsh conditions during the processing of the product which may account for its presence in exposed samples. The production of spores enables this organism to withstand unfavourable conditions such as low temperatures or heat and may improve the chances of Bacillus to be present in high numbers in the air [33]. The most common airborne Bacillus was, for many years, dismissed as harmless contaminants with weak to non-existent pathogenicity. However, infections are increasingly reported and because the spores are abundant in the environment and usual methods of disinfection and antisepsis are powerless in controlling them, these organisms are becoming a serious health risk [34]. Certain strains of Bacillus are known causative agents of food poisoning and intoxication and their isolation from garri, which most times requires little or no cooking, is a cause for concern. Several dried food samples have been reported to contain some of these organisms [35],[36]. *Staphylococcus aureus* is found in all individuals and usually expelled from the respiratory tract through the skin, nose and mouth which may also account for their presence in the post processed product. Various researchers [36-39] have reported that the presence of *Staphylococcus aureus* in food is an indication of environmental and human contamination. The practice of leaving garri open for sales, and the regular unhygienic practice by buyers who using their bare hands fetch garri to taste during buying could have been the sources of *Staphylococcus aureus* found in the sampled garri. *Yersinia* spp isolated from the market is contrary to other works of Orji *et al.* [20] and Olapade *et al.* [17]. The main reservoir of *Yersinia* spp is pigs explaining that its presence in one of the garri samples indicates cross contamination by buyers who after buying pork did not wash their hands before touching.

5 Conclusion

The heavy bacterial contamination and vast array of bacteria isolated from the garri sold in both markets portend alarming danger posed by consumption of garri sold in the markets. This is likely to be associated with poor post processing, handling practices such as spreading on the floor, mat and sometimes on high density polyethene, after frying to allow it to cool before sieving into finer grains and the open display in bowls and basins in the market, measurement with the aids of bare hands, coughing and sneezing while selling and the use of non-microbiologically determined hessian bags for packaging and haulage. Therefore renewed vigilance on the efficacies of food processing conditions, handling techniques and handlers technical knowhow, hygiene practices and safety of finished products are hereby recommended. Also, good sanitary practice should be enforced concerning the sale of cassava based foods. Personal hygiene of hawkers and sanitation of utensils are important. Sellers should be enlightened on hygienic practices. For effective checkmating, strict application and implementation of quality control, quality assurance, good manufacturing practice and the hazard analysis critical control point principles will help to ensure the safety of Garri consumed by several people who consume garri sold in both Nkalagu and Okwor markets, Nigeria and Africa in general. Buyers should be discouraged from touching displayed garri with their bare hands.

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