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Bacteriological Quality Evaluation and Safety of Randomly Selected Ready-to-Eat Foods Sold in Port Harcourt City, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author FSI designed the study, provided technical expertise, performed the statistical analysis and proofread the manuscript. Author VTI wrote the protocol and managed the analyses of the study. Author FSI wrote the first draft of the manuscript and both authors managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Ready-to-eat (RTE) foods are composite foods sold to consumers for consumption which do not require significant further processing except re-heating or completion of cooking process. These foods may constitute likely potential hazard to human health due to non compliance with food safety regulations by food handlers. This study was aimed at evaluating the bacteriological quality of ready-to-eat foods sold by selected food vendors in Port Harcourt city. Bacteriological analyses were conducted on 15 samples which included jollof rice, okro soup, egusi soup, jollof beans and porridge yam. Assessment of the possible bacteria in the food samples were separately performed using pour plate technique on various media for isolation and enumeration of the bacteria population in the samples. Bacteria isolates were identified based on colonial morphology,

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microscopy and biochemical tests. The standard plate counts obtained were compared to the bacteriological guidelines and the specifications by International Commission for Microbiological Specification for Foods (ICMSF). Data were analysed using the one way ANOVA and post-hoc Scheffe test. Bacterial growth was present in all the food types and bacterial counts differed remarkably among the various food samples investigated in the study. The mean total aerobic plate count ranged from 4.4×10^7 cfu/g to 8.2×10^7 cfu/g in all the food samples. The result indicated high levels of mean total aerobic count in Jollof beans $(8.2 \times 10^7 \text{ cfu/g})$, followed by jollof rice $(6.6 \times 10^7 \text{ cfu/g})$ cfu/g) while porridge yam had the lowest count (4.4×107 cfu/g). There is significant difference (p≤0.05) between the bacterial loads in the food samples investigated. The bacterial isolates detected in the food samples were Staphylococcus aureus (100%), Escherichia coli (100%), Salmonella sp (73.3%) and Vibrio sp (6.67%). The results revealed that the bacteriological parameters analyzed exceeded recommended limits and this level of contamination of the RTE foods were not of acceptable quality and safety. The consumption of these foods could portend a risk of foodborne diseases and other health challenges. Therefore, this result is intended to draw the attention of relevant authorities to ensure that adequate hygienic standards and regular monitoring of the quality of RTE foods are improved and practiced to avoid possible foodborne infections.

Keywords: RTE foods; food contamination; foodborne pathogens; food borne infections.

1. INTRODUCTION

Food is one of the most vital nutrients for the promotion of human health and prevention of disease. It is regarded as one of human primary needs besides clothing and housing. Due to the vital role of food in human existence, it is imperative to maintain high level of food safety in order to ensure that human being is safe from diseases or other related health hazards associated with food [1]. Diseases that result from foods are one of the major health problems in developing and developed countries [2]. Conditions of food safety include efforts to avoid contamination from biological, chemical agents and other substances that can endanger human health [1]. The American CDC reported that about 77% of food poisoning occurs in restaurants, 20% in homes and 3% from commercial foods relating to non-compliance with food standards and secondary pollution [3]. Ready-to-eat foods can be defined as foods and beverages prepared and/ or sold by vendors on the street and in other public places for immediate consumption or consumption at a later time without further processing or preparation [4,5,6]. Due to socio-economic changes characterized by increased mobility, resulting in more ready-to-eat foods taken outside the home, food vendors services are on the increase and responsibility for the food safety have been transferred from individuals/families to the food vendors who rarely enforce good manufacturing practices [7]. Street foods are frequently linked with gastrointestinal diseases such as diarrhea and typhoid fever due to improper handling and

serving practices [8-11]. According to Nkere et al. [9] poor environmental sanitation is largely responsible for much of the contamination and poor personal hygiene among the food handlers. These bacteria can come in contact with the foods when they are prepared especially in unhygienic environments and contaminated cooking utensils [12,13]. Several studies showed that different pathogens have been isolated from ready-to-eat foods in different countries which include Staphylococcus aureus, Escherichia coli, Salmonella sp, Shigella sp, Klebsiella sp, Pseudomonas sp, Vibrio sp, Campylobacter sp and Listeria monocytogenes [1,3,10,14-24]. The aforementioned observations confirm the risk posed by consuming these vended foods. According to Food Codex Commission of World Health Organization classification, cooked readyto-eat foods are among the high risk foods [3]. classification Similarly, Stewart and Humphrey [25] attributed the cases of food infection and intoxication to poor and inadequate sanitary condition observed in processing of many locally made foods.

In Nigeria, hawking of ready-to-eat foods in streets and markets even along the road for travelers is very common. These food vendors enjoy huge patronage from different societal classes. Unfortunately, none of these food hawkers or vendors is licensed or monitored by relevant agencies saddled with the responsibility of ensuring the safety of our foods. Thus, owing to the manner and conditions these vendors operate, there is possibility that some of the ready-to-eat foods may be contaminated by foodborne pathogens. Therefore, this study was conducted to evaluate the bacteriological quality and safety of street-vended ready-to-eat foods in Port Harcourt Metropolis. This study was undertaken due to the position of ready-to-eat foods has occupied in our society and the associated health issues in their consumption. However, such foods have been implicated in foodborne illnesses and diseases that remains a major public health challenge. The work will benefit the unsuspecting consumers, government health agencies and the vendors on any health risk such food might pose.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of fifteen (15) ready-to-eat food samples were collected randomly from vendors in Rumuokoro areas of River state and five samples were collected from each seller. Rumuokoro is a town in Obio-Akpor local Government Area, Rivers state, Nigeria, located at 4°51 "6'N, 7°2 50'E. The ready-to-eat food samples comprised of jollof rice, okro soup, 'egusi' soup, jollof beans and porridge yam. They were properly coded, packaged separately in sterile containers and transported in cold pack to Food and Industrial Laboratory of the Department of Microbiology, University of Port Harcourt within 1 h for prompt processing.

2.2 Bacteriological Analysis of the Food Samples

2.2.1 Sample preparation, culture and bacterial count

The modified method of Akoachere et al. [26] was used for the preparation of the samples. Twenty five grams of each food sample was weighed and homogenized by blending in 225 mL of sterile buffered peptone water. Thereafter, one millilitre of each food sample homogenate was mixed into 90 mL of the buffered peptone water in a test tube. Serial dilution was made to 10^5 in five other test tubes comprising 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6} . A 0.1 aliquot portions of each of the diluted samples were spread onto duplicate sterile plates of Nutrient agar, Eosin Methylene Blue (EMB) agar, Mannitol Salt agar (MSA). Thiosulphate Citrat Bile-salt Sucrose (TCBS) agar and Xylose Lysine Deoxycholate (XLD) agar, for total aerobic count, Escherichia coli, Staphylococcus aureus, Vibrio sp, and Salmonella sp, respectively. The inoculated plates were incubated aerobically at 37°C for 24 h. After incubation time, the different culture plates were examined for bacterial growth and discrete colonies were counted using the colony counter (Gallenkamp, England) and expressed as colony forming units per gram (CFU /g) of sample homogenate.

2.2.2 Identification of isolates

Pure cultures were obtained and stored on nutrient agar slants at 4℃ pending identification. The isolates were confirmed based on cultural characteristics and biochemical tests which include IMViC test, carbohydrate utilization, and reaction on Tri-Sugar Iron (TSI) medium, starch hydrolysis, gelatin liquefaction, nitrate reduction, oxidase, urease production and motility test. The bacterial isolates were identified by comparing their characteristics with Bergey's Manual of Determinative Bacteriology [27].

2.3 Statistical Analysis

Statistical analysis of data was carried out using one-way analysis of variance (ANOVA) and posthoc Scheffe tests were used to analyse the level of contamination according to type and source of ready-to-eat foods at P = .05 level of significance using SPSS version 20 package. Windows Excel program was used to draw all the graphs and calculate the percentage occurrence of pathogens in the samples.

3. RESULTS AND DISCUSSION

The occurrence and levels of the bacterial pathogens varied significantly (P = .05) with the type of sample and sampling site. The results of the present study indicated that the bacteria isolated from all the food types and samples collected include S. aureus, E. coli, Salmonella sp and Vibro sp. The isolation of S. aureus, E. coli, Salmonella sp and Vibro sp. corroborate with previous findings in which these organisms were isolated from ready-to-eat foods [1,8,9,22,24,28-33]. Moreso, previous studies indicated that different pathogens have been isolated from ready-to-eat foods including E. coli, Salmonella spp. and Campylobacter confirming the risk posed by consuming the foods [34]. The presence of coliform bacteria (E. coli) in all the food samples investigated in this study could be from preparation, handling practices and feacal contamination of the ready-to-eat food samples. This assertion had previously been proposed in other reports [24,30,35]. Distribution of mean

bacterial counts by sample location and type of food samples investigated is shown in Table 1. The result showed that jollof beans from Miss A had the most aerobic count $(10.5 \times 10^5 \text{ cfu/g})$, while jollof rice and okro soup both from Mrs B had the least total aerobic count $(4.7 \times 10^5 \text{ cfu/g})$. The results are similar to the findings of Bukar et al. [36], Miriam et al. [37], Wogu et al. [30]. Clarence et al. [14] and Chukwu et al. [19] have attributed the presence of heterotrophic bacteria in foods to the unhygienic water, food products and utensils used in the production and packaging of the food samples. Viable counts of E. coli was highest in jollof beans obtained from Miss A, with the least viable E. coli count obtained in the porridge yam sample from Mrs. B. Staphylococcal counts recorded in jollof beans from Miss A was the highest among the food sampled (8.6 x 10^5 cfu/g), and the least *Staphylococcal* count was observed in the porridge vam sample gotten from Mrs. B (3.1 x 10^5 cfu/g). The jollof beans sample from Miss C had the highest Salmonella count (5.7 x 10⁵ cfu/g), with jollof rice and egusi soups samples from Miss A having the least Salmonella count (3.1 x 10⁵ cfu/g). Salmonella sp was not detected in jollof rice, jollof beans, porridge yam and egusi soup samples from Mrs. B and Miss C. Vibrio sp was only isolated in jollof rice sample obtained from Miss A (3.1 x 10^5 cfu/g). There is significant difference (P = .05) between the bacteria loads in the different food samples among the individual food vendors. This may be due to the level of hygiene observed by the vendors during food

preparation, location and operations during selling as well as the source of water used in cooking and washing of utensils.

The heavy growth of these pathogens are expected because the points where the foods are vended were open to different sources of contamination like car exhausts, rising dusts and littered or heaps of garbage which provide a hiding place for insects and animal pests [15,38]. The finding of this study is strongly worrisome as known food pathogens were isolated from all the food samples evaluated and all marginally exceeded the International Commission for Microbiological Specification for Foods [39] which stipulates that ready-to-eat foods with plate counts between $0 - 10^3$ is acceptable, between $10^4 - \le 10^5$ is tolerable and 10⁶ and above is unacceptable. Therefore, all the foods investigated in this study are unacceptable in all ramification based on Nigerian and International Standards for foods. This situation invariably puts the health of the public who innocently consume these foods at risk. Street foods are seen to constitute a major public health risk because of absence of basic infrastructure and services, difficulty in curtailing the large numbers of vending operations as a result of their diversity, mobility and temporary nature [40]. Food handling personnel play important role in ensuring food safety throughout the chain of food production, processing, storage and preparation [33]. According to previous reports [41-43], mishandling and

 Table 1. Distribution of mean bacterial counts by sample location and type of food samples investigated

Source	Sample	(10 ⁷ cfu/g)		Viable cou	nts (10 ⁵ cfu/g)	
		NA	EMBA	MSA	XLD	TCBS
Miss A	Jollof rice	6.1±0.6	4.4±0.43	5.8±0.07	3.1±0.75	3.1±0.55
	Okro soup	5.1±0.9	5.9±0.6	3.9±0.12	3.6±0.27	ND
	Egusi soup	7.3±0.17	6.0±0.77	4.7±1.2	3.1±0.45	ND
	Jollof beans	10.5±0.7	7.0±1.2	8.6±0.93	4.4±0.85	ND
	Porridge yam	5.1±0.21	6.0±0.56	8.1±0.58	3.6±0.66	ND
Mrs B	Jollof rice	4.7±0.77	4.4±0.67	4.7±0.89	ND	ND
	Okro soup	4.7±0.34	4.4±0.92	4.7±0.7	3.2±0.25	ND
	Egusi soup	5.1±0.56	6.0±0.44	4.3±0.5	4.3±0.32	ND
	Jollof beans	8.1±0.18	4.4±0.34	4.9±0.22	ND	ND
	Porridge yam	3.9±0.14	3.1±0.26	3.1±0.42	ND	ND
Miss C	Jollof rice	8.8±0.17	6.3±0.72	6.6±0.26	3.6±0.24	ND
	Okro soup	7.5±0.9	6.0±0.53	5.1±0.58	4.4±0.16	ND
	Egusi soup	5.8±0.24	6.0±0.42	5.6±0.32	ND	ND
	Jollof beans	6.1±0.22	5.9±0.02	3.9±0.22	5.7±0.4	ND
	Porridge yam	4.1±0.16	6.7±0.33	5.1±0.26	4.1±0.54	ND

Keys: NA: Nutrient agar, EMBA: Eosin Methylene Blue Agar, MSA: Mannitol salt agar, XLD: Xylose Lysine Desoxycholate agar, TCBS: Thiosuphate citrate bile salt agar, ND: Not detected disregard to hygienic measures on the part of the food vendors have been reported to introduce contaminants and pathogens that survive and multiply in sufficient numbers to cause illness in the consumer. The preparation of foods in advance of consumption, exposure and holding of food at ambient temperature makes it conducive for microbial multiplication coupled with the rich nature of jollof beans could equally be a factor in the increased microbial loads of the samples as previously reported [33,44,45].

Table 2 depicts the average mean bacterial population of the ready-to-eat food samples. The mean total aerobic plate count ranged between 4.4×10^7 cfu/g (porridge yam) and 8.2×10^7 cfu/g (jollof beans), coliform (E. coli) ranged between 5.0×10^5 cfu/g (jollof rice) and 6.0×10^5 cfu/g (egusi soup), Staphylococcus aureus ranged between 4.6×10^5 (okro soup) and 5.8×10^5 (jollof beans). Salmonella sp ranged between 3.4×10⁵ (jollof rice) and 5.1×10^5 (jollof beans). Vibro sp was only isolated from jollof rice sample from Miss A (3.1×10⁵ cfu/g). The bacterial count obtained in our finding is comparable to that reported by [32] who reported total aerobic plate count range of $1.7 \times 10^3 - 7.1 \times 10^9$ cfu/g and coliform range of 2.3×10^7 cfu/g. The finding of this study differed with the study conducted by [33] who obtained lower total aerobic count and coliform count of 2.5×10³ cfu/ml for rice to 9.1×10^{6} for coleslaw and 3.2×10^{3} for jollof rice to 3.4×10^4 for fried rice and coleslaw, respectively. In recent study on packaged fried rice, mean viable counts of heterotrophic bacteria, coliform bacteria and Salmonella-Shigella bacteria were reported to be 42×10^5 cfu/g, 23×10^3 and 14×10^3 cfu/g, respectively [24]. WHO [46] have associated high contamination of coliforms in foods with symptoms like nausea, vomiting, retching, abdominal cramp, diarrhea and prostration. The mean total aerobic count obtained in this study is higher than that reported by Wogu et al. [30] who observed a total colony count range of 2.0×10^4 - 1.2×10^6 cfu/g for bacteria in ready-to-eat rice from local fast food centers

and standard fast food centre in Benin City. Our result also differ in mean microbial range of 3×10^3 - 5×10^3 cfu/g and 2.3×10^4 - 3.8×10^4 cfu/g, 8×10^3 - 1.5×10^4 cfu/g and 7×10^3 - 2.8×10^4 for meat pie in standard eatery, air preserved, refrigerated and local kiosk, respectively.

The finding of this study is in agreement with studies by [15] who reported presence of E. coli in RTE beef curry. Some other studies have reported the absence of Salmonella in RTE food samples [15,47,48]. The presence of Salmonella in the present work showed that good handling practices during the cooking process and good storage facilities were not available for RTE. The detection of Staphylococcus aureus in the food samples is in contrast with the reports of [15] and [49], who reported that no Staphylococci were detected in RTE meal samples in Malaysia and Sao Paulo, Brazil, respectively. In the study by [48] in UK on vegetable salad samples and sauce samples used in salad preparation indicated that 4.7% and 5% of the respective samples were not acceptable and confirmed presence of Staphylococcus sp. and E. coli. Similarly, Tavakoli et al. [3] detected E. coli and Staphylococcus aureus in ready-to-eat food in four military restaurants in Tehran.

The percentage occurrence of the bacterial isolates is presented in Fig 1. The result revealed that Escherichia coli and Staphylococcus aureus were present in all the various food samples (100% each) whereas Salmonella sp occurred in eleven samples (73.3%). Vibrio sp was only observed in only one out of the 15 samples accounting for 6.67%. The occurrence of E. coli and Staphylococcus aureus in all the food samples may be as a result of poor handling and keeping method by the food vendors. Similar results have been obtained in previous studies [30,36]. However, Mba et al. [31] have reported a percentage occurrence of 33.4% for coliform bacteria and 27.3% for Staphylococcus aureus in a study on the bacteriological status of five selected street vended cooked foods in Calabar.

Sample	10 ⁷ cfu/g	10 ⁵ cfu/g					
	aerobic count	E. coli	S. aureus	Salmonella sp	Vibro sp		
Jollof rice	6.6±0.51	5.0±0.60	5.7±0.41	3.4±0.50	3.1±0.55		
Okro soup	5.8±2.04	5.4±0.68	4.6±0.47	3.7±0.23	ND		
Egusi soup	6.1±0.32	6.0±0.54	4.9±0.68	3.7±0.39	ND		
Jollof beans	8.2±0.32	5.8±0.52	5.8±0.46	5.1±0.43	ND		
Porridge vam	4.4±0.17	5.3±0.38	5.4±0.42	3.9±0.60	ND		

Table 2. Average mean bacterial population of the ready-to-eat food samples

ND – Not detected



Bacterial isolates

Fig. 1. Percentage occurrence of isolates in all the food samples

The results are contrary to the findings of [18] who reported low prevalence of Staphylococcus aureus (3.2%) in street vended foods. Our results collaborate with the findings of [50] and [51] who reported equally high prevalence of Staphylococcus aureus in street-vended food. The sources of bacterial contamination of these foods may likely differ. It is most likely that the presence of coliform (E. coli) indicates possible feacal contamination of the food vendors, water and other utensils as well as poor hygienic processing practices of the food vendors [3,31]. Bukar et al. [36] reported higher occurrence of Vibrio cholera (15, 25%) in minimally processed and fully processed food samples. In a study conducted by [6], Staphylococcus aureus and E. coli were detected in 9% and 3% of 326 street vended foods in Korea with the mean value of 3.75±0.56 log cfu/g and 2.33±0.90 log cfu/g, respectively. According to [30] the frequency of occurrence of E. coli and Staphylococcus aureus in ready-to-eat rice sold in Benin City was 37.5% each. Our observation is in contrast with their parahaemolyticus Vibrio report that and Salmonella spp were not detected in any of the food samples.

S. aureus is a normal flora of the human skin, nasal passage and throat of most healthy people and may have entered the food chain through such sources which suggests poor hygiene practices of the operators. When *Staphylococcus aureus* is permitted to grow in foods, it can produce a toxin that causes illness and may be destroyed by heat but its toxin is heat stable. The presence of these organisms could cause mild to

severe symptoms of diseases such as diarrhea. typhoid and cholera [31,37]. According to [52], Salmonella sp and Staphylococcus aureus are the most common foodborne pathogens and are responsible for food poisoning and food associated infections. According to [40], the contamination of street food due to Salmonella sp. can be explained by the use of dirty dishwater (from dirty dishes) or lack of good hygiene practices of vendors when handling street food. Manguiat and Fang [53] reported that contamination of street food in the Philippines was mainly due to S. aureus, Salmonella sp. and Vibrio cholerae, while Salmonella sp. was isolated in 15% of samples from grilled pork and chicken. Oghene et al. [22] in separate study reported the contamination of street food and vegetables in Enugu state, Nigeria by S. aureus, Bacillus cereus, Vibro spp, Salmonella spp, E. coli and Shigella sp. It is curious that the high level of food contamination observed in the present study had also been reported earlier in Nigeria and other parts of developing countries [3,9,17,20,22-24,54-55]. This trend of event has been linked to poverty and low standard of personal and environmental hygiene in these regions [9,10]. This critical situation calls for stricter public health regulations and implementation of food sanitation practices regarding the sale of foods on streets by food vendors. In order to minimize this time bomb in our society, there should be adequate provision of portable water, training and educating the food vendors on safe and good hygiene practices especially hand washing and enforcement of legislations on food

handling and processing as well as environmental sanitation.

4. CONCLUSION

The findings of this study of the ready-to-eat foods sampled revealed high bacterial load in all the food samples and showed that the total aerobic colony counts, coliform (E. coli) counts and Salmonella count were above the acceptable limit. The result obtained indicated that the ready-to-eat foods were contaminated with different bacterial pathogens such as Staphylococcus aureus, Escherichia coli. Salmonella sp and Vibro sp. The presence of these food pathogens in the foods could pose a serious public health hazard to unsuspecting consumers as all these bacterial pathogens have been implicated in food borne illnesses and diarrheal diseases. The detection of these organisms in all the ready-to-eat foods investigated portends danger and could be associated with poor personal hygiene, poor food preparation, lack of good manufacturing practices and non compliance of Hazard analysis and critical control points (HACCP) principles during the preparation, packaging and serving of these foods to consumer. In order to minimize or arrest this unwholesome and ugly trend of readyto-eat food contamination, it is imperative for relevant agencies in public health and food safety to organize training and teaching on food safety and hygiene for food vendors as well as strictly implement existing legislations to ensure the safety of these commercially vended foods. Moreso, the following primary food Safety measures should be effectively observed by food handlers and vendors: proper hand washing practice, preparation and selling of foods in hygienic premises, proper covering of prepared foods, washing of utensils and dish with soap, use of portable water as well as proper disposal of wastes among others.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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