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MICROBIOLOGICAL QUALITY OF READY-TO-EAT FOODS SOLD IN MILE 3 MARKET, PORT HARCOURT, RIVERS STATE

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Abstract

Ready-to-eat (RTE) foods sold in most markets are occasionally contaminated with harmful pathogenic bacteria. The primary cause of foodborne infections is the presence of pathogenic bacteria that produce viable toxins in food, which is of public health concern. Sixty (60) samples of RTE foods comprising Suya meat, Meat pie Moi moi and Egg roll were randomly obtained from retailers in Mile 3 Market, Port Harcourt, Rivers State. The samples were subjected to standard microbiological procedures for total heterotrophic bacterial count (THBC), *Salmonella*, total coliform, faecal coliform and *Staphylococci* counts. THBC of the food samples ranged from $1.5\pm0.1 \times10^6$ to $3.7\pm0.1 \times10^6$ CFU/g. The THBC of the meat pie and egg roll was significantly ($P<0.05$) higher than the THBC of other food samples. The *Salmonellae,* total Coliform, faecal Coliform and *Staphylococcal* counts of the food samples ranged from $2.2\pm0.4\times10^5$ to $3.2\pm0.1\times10^5$, 1.5 ± 0 to $2.0\pm0.8\times10^5$, 1.3 ± 0.8 to $1.8\pm1.1\times10^5$, and 2.1 ± 0.4 to $2.7\pm0.2\times10^5$ CFU/g. *Salmonellae* count of egg roll sample showed significant difference ($P\leq0.05$) compared to other RTE foods. Also, there was no significant differences ($P\geq0.05$) in the total coliform, faecal coliform and *staphylococci* counts of the food samples. *Staphylococcus, Bacillus, Escherichia coli, Shigella, Pseudomonas putida, Vibrio, Cronobacter, Klebsiella* and *Arthrobacter* spp were isolated. Contamination above 10^6 CFU/g food and the presence of potential foodborne pathogens could be risky. There is need for consumer's awareness on the dangers of consuming contaminated foods, education of food vendors on food hygiene and constant monitoring of ready-to-eat foods in order to prevent the outbreak of food-borne illnesses and food safety.

KEY WORDS: Bacteria, Coliforms, Contaminated foods, HACCP, Ready-to-eat foods.

1.0 Introduction

Ready-to-eat (RTE) food, also known as fast food, is usually bought from a place that provides affordable costs, prompt service, and convenience (Osakue *et al.*, 2016). These dishes are easy to prepare and easily accessible from roadside sellers, hawkers, and shops, people find them more interesting

without questioning their safety (Sudad, 2018). Ready-to-eat foods have been reported to be readily available, affordable, and provide diverse/variable food source and employment; and with a potential for improving food security, nutritional status and general social security (Draper, 1996). It is however, a veritable source of food borne pathogen (Mensah, 2002). Over 600 million foodborne illness cases and 420,000 fatal infections are caused by bacterial contamination each year (WHO, 2015). According to Addis and Sisay (2015), the primary cause of foodborne diseases is the presence of pathogenic bacteria that create toxins in food, particularly in foods originating from animals. These ailments present serious health risks that, if consumed, could potentially result in death (Obande *et al.*, 2017). Consumers of RTE foods mainly consider convenience or ease of access rather than quality, safety and hygiene aspects (Bakobie *et al*., 2017). Food safety is crucial in achieving better human nutrition through healthy nutritious diets. Improving food safety is thus a key in achieving Sustainable Development Goal number 3, which is good health and wellbeing. This study aims to investigate microbiological quality associated with ready-to- eat foods like Meat pies, Moi moi, Suya meats, and egg rolls sold in Mile 3 market, Port Harcourt, Rivers State in order to ascertain the safety of these foods, given the widespread consumption of the different ready-to-eat foods by the public.

2.0 Material and methods

2.1 Study area

The study was carried out in Mile 3 Market, Port Harcourt, Rivers State, situated in the South-South (Niger Delta) region of Nigeria, along the Bonny River. Port Harcourt is located between Latitude 4.75°N and 4.95°N and Longitude 6.95°E and 7.10°E. The city has an area of 369 km² It is characterized by a tropical climate with two distinct seasons: a rainy season (April-October) and a dry season (November- March), based on the vegetation classification of Nigeria.

2.2 Sample collection

A total of sixty (60) ready-to-eat food samples of Meat pies, Moi moi, Suya meat and Tiger nut juices were aseptically and randomly obtained from retailers in Mile 3 Market in Port Harcourt, Rivers State. Twenty (20) samples of each food type were obtained from the different sampling points at monthly interval for three (3) months (February, March and April, 2024). The samples were randomly collected and packaged in a sterile transparent zip-lock polypropylene bags containing ice block and transported to the microbiology laboratory within 1 hour of purchase.

2.3 Sample analysis

Sample analysis was conducted within 3 hours of sample collection. Ten (10) grams of each food sample were macerated using a sterile marble mortar and pestle; and homogenized in 90ml sterile normal saline in sterile test tubes. The test tubes were shaken thoroughly, ten (10) fold serial dilutions of the resultant homogenates were made to obtain 10^{-2} to $10^{-5}$and 10^{-2} to 10^{-4}

respectively. one (1) mL was taken from the first test tube and added to the second test tube and mixed thoroughly, and was continued until the tenth serial dilution. Each serial diluted sample of 0.1ml was transferred to the following media; Nutrient agar (NA), MacConkey Agar (MCA), Eosin methylene blue agar (EMB), *Salmonella-Shigella* agar (SSA) and Mannitol salt agar (MSA), respectively using a spread plate method (Cheesbrough, 2006). All the bacterial plates were incubated at a temperature of 37^{o} C for 24 hours. Bacterial colonies found on each plate were counted using a colony counter.

Distinct morphological properties of colonies were observed and characteristic colonies were isolated and purified by repeated sub-culturing on Nutrient agar for further identification. Tentative identification of isolates was done by Gram staining, citrate utilization, indole production test, methyl red test, vogues-proskauer test, oxidase, catalase, coagulase, sugar fermentation; triple sugar test (TSI), motility, and starch utilization (Hi-media manual, 2003). Confirmatory identification of the bacterial isolates was based on standard biochemical methods (Jolt *et al*., 1994).

2.4 Data analysis

Data analysis was performed by computing the means and standard deviations of the results obtained using Microsoft Excel 2016 (Microsoft Corporation). One-way Analysis of Variance (ANOVA) was used with the Statistical Package for Social Science (SPSS) software version 20 (IBM Corp, Armonk, NY, USA) to calculate and compare the means. Duncan Multiple Range test was used to separate the means. Significance tests was considered statistically significant when *p* values significant level set at < 0.05.

3.0 Results

3.1 Bacterial mean Counts of Samples

The microbiological quality of food samples on the bases of bacterial counts are presented in Table 1. Results showed that the total heterotrophic bacterial count (THBC) of the food samples ranged from 1.5±0.1 to 3.7±0.1 ×10^{6} CFU/g. The THBC of meat pies (MP) and egg roll (ER) was significantly (P<0.05) higher than the THBC of other food samples. The *Salmonellae* count ranged from 2.2±0.4 ×10^{5} CFU/g to 3.2±0.1×10^{5} CFU/mL. There was significant difference (P≤0.05) in the *Salmonella* count of ER compared to other food samples. Total coliform count ranged from 1.5±0.6 to 2.0±0.8×10^{5} CFU/g, faecal coliform count ranged from 1.3±0.8 to 1.8±1.1×10^{5} CFU/g and *staphylococcal* count of the food samples ranged from 2.1±0.4 to 2.7±0.2×10^{5} CFU/g. There were no significant differences (P≥ 0.05) in the total coliform, faecal coliform and *staphylococci* counts of the food samples.

Based on their phenotypic (colonial, morphological and biochemical) characteristics, a total of thirty-six (36) bacterial isolates were identified, which belonged to nine (9) genera. These were; *Arthrobacter, Escherichia, Shigella, Pseudomonas, Cronobacter, Vibrio, Staphylococcus, Bacillus* and *Klebsiella.* These isolates displayed similar biochemical and morphological characteristics with those on the ABIS data base.

3.2 Distribution of bacterial isolates in food samples

The distribution of bacterial isolates from food Samples analysed is presented in Table 2. Results showed that the highest bacterial distribution and occurrence was recorded in Meat pie (MP) followed by Egg roll (ER), Suya meat (SM) and Moi moi (MM) samples.

3.3 Percentage frequency of biofilm production and haemolysis of bacterial isolates

The percentage frequency of biofilm and haemolysis production ability of the identified bacterial isolates is presented in Table 3. Results revealed that 100% of *Escherichia coli* were positive for biofilm production and haemolysis production.

3.4 Prevalence of bacterial isolates recorded in food samples

The prevalence of bacterial isolates recorded in food samples analysed are presented in Fig. 1. Results showed that *Staphylococcus* sp had the highest occurrence (22.2%) followed by *Bacillus* sp (19.44%), *Shigella* sp (13.89%), *Escherichia* sp (8.33%), *Pseudomonas* sp (8.33%), *Cronobacter* sp (8.33%), *Vibrio* sp (8.33%), and *Klebsiella* sp (8.33%); while *Arthrobacter* sp was the least (2.78%) occurring bacterial isolate.

Table 1: Mean Bacterial Counts (CFU/g) from Ready to Eat Foods Sold in Mile 3 Market

Sample	THBC ($\times10^6$)	*Salmonellae* ($\times10^6$)	Total coliform ($\times10^6$)	Faecal coliform ($\times10^6$)	*Staphylococci* ($\times10^6$)
Moi Moi	1.5±0.1 [a]	2.2±0.4 [a]	2.0±0.8 [a]	1.3±0.8 [a]	2.1±0.4[a]
Meat Pie	3.7±0.1 [b]	2.4±0.2 [b]	1.8±1.3 [a]	1.8±1.1 [a]	2.5±0.1 [a]
Suya Meat	1.7±0.1 [a]	2.6±0.6 [c]	1.5±0.6 [a]	1.6±0.6 [a]	2.7±0.2 [a]
Egg Roll	3.4±0.4 [b]	3.2±0.1 [d]	1.8±0.3 [a]	1.8±0.3 [a]	2.2±0.5 [a]
P-value	0.001	0.001	0.93	0.93	0.30

*Means with similar superscript (a,b,c) down the group showed no significant difference (P>0.05)

Keys: CFU/g= Colony forming units per gram, **THBC**=Total Heterortophic Bacteria count

Table 2: Distribution of Bacteria Isolates from Food Samples Analysed

Food Sample	Organisms Isolated
Moi moi	*Bacillus cereus*, *Shigella sonnei*, *Escherichia coli*, *Vibrio* sp, *Staphylococcus aureus*, *Klebsiella* sp, *Staphylococcus* sp, *Cronobacter* sp.
Meat Pie	*Staphylococcus aureus*, *Bacillus cereus*, *Shigella* sp, *Escherichia coli*, *Athrobacter* sp, *Shigella sonnei*, *Pseudomonas putida*, *Cronobacter* sp *Staphylococcus* spp, *Bacillus* sp

Suya Meat	*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* spp *Shigella sonnei*, *Pseudomonas putida*, *Bacillus cereus*, *Vibrio* sp, *Cronobacter* spp, *Bacillus* sp.
Egg roll	*Klebsiella aerogenes, Bacillus cereus*, *Staphylococcus* sp, *Escherichia coli*, *Shigella* sp, *Pseudomonas putida*, *Athrobacter* spp, *Vibrio* sp *Staphylococcus aureus*

Table 3: Percentage Frequency of Biofilm Production and Haemolysis of Bacterial Isolates

Isolates	Biofilm test (%)	Haemolysis test (%)
***Bacillus* spp (6)**	4(66.7)	2(33.3)
***Klebsiella* spp (4)**	2(50)	2(50)
***Cronobacter* spp (2)**	2(100)	0(0)
***Arthrobacter* sp (1)**	1(100)	0(0)
***Staphylococcus* spp (7)**	3(42.9)	2(28.6)
***Pseudomonas* spp (4)**	3(75)	4(100)
***Vibrio* spp (5)**	2(40)	2(40)
***Escherichia* spp (3)**	3(100)	3(100)
***Shigella* spp (4)**	2(50)	2(50)

Keys: % percentage

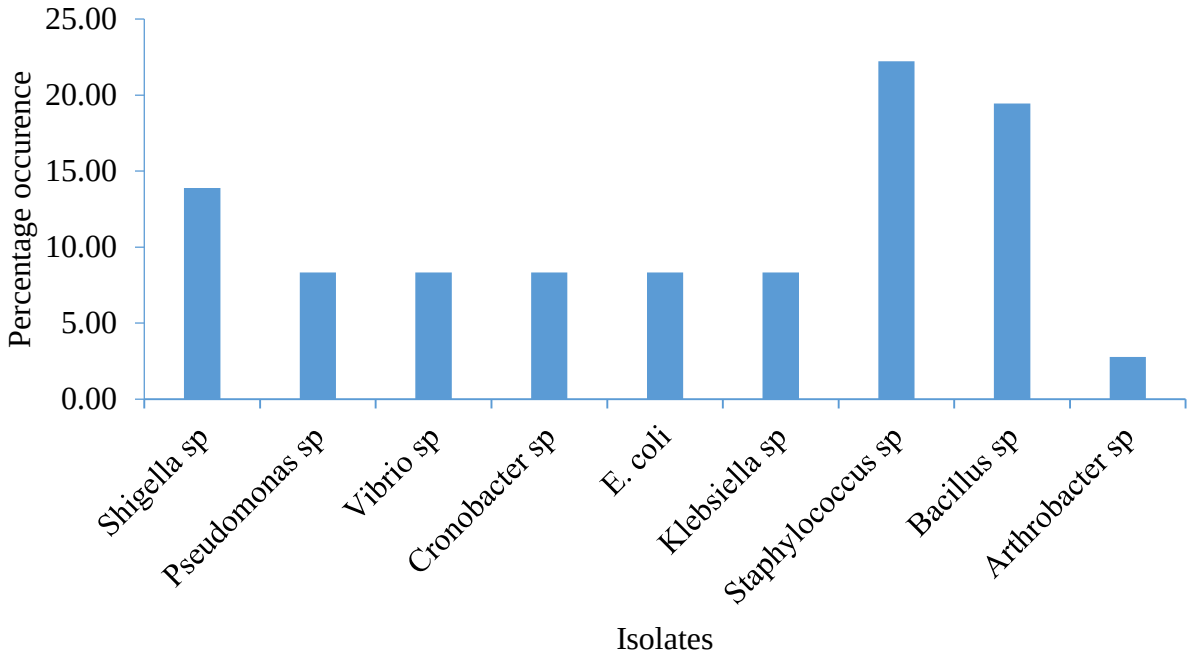

Fig. 1: Prevalence of the bacterial isolates in all the samples analysed

4.0 Discussion

Ready-to-eat foods sold in markets are widely consumed in Nigeria, but their safety is of concern due to the risk of bacterial contamination. The microbiological quality of ready-to-eat foods sold in Mile 3 Market Port Harcourt, Rivers State was investigated with the aim of understanding the prevalence of some pathogenic bacteria associated with these foods and the public health implication. Ready-to-eat foods are easy to make and easily available from retailers, street/roadside vendors and hawkers, hence people find them more interesting without questioning their safety (Sudad, 2018). Some ready-to-eat foods play a pivotal role in and harboring the transmission of a diverse range of pathogenic bacteria that may cause foodborne illnesses. Studies reveal that some ready-to-eat foods sold in markets are contaminated beyond acceptable microbiological limits, and some are within acceptable microbiological standard (Akindele and Ibrahim, 2016).

Total heterotrophic bacterial count (THBC), *Salmonella*e, total coliform, faecal coliform and *staphylococcal* were enumerated. The THBC of meat pie and egg roll was significantly ($P<0.05$) higher than the THBC of other food samples. World Health Organization, (2006) guidelines for ready-to-eat foods provides THBC of 10^6 CFU/g as signify satisfactory and acceptable whereas any ratio above 10^6 CFU/g is considered unsatisfactory. Hence the mean bacterial counts of some of the food sampled (10^6 CFU/g), in this study were within the acceptable range of World Health Organization (WHO) RTE food standard. The high microbial load recorded for meat pie could be associated with the production and handling of the product. Moi moi is often wrapped in dried nylon and some in

aluminum foil that are not cleaned to remove microbial load (Okeke *et al*, 2008). Left over products are normally re-heated for subsequent day sales.

The study showed that different food samples were contaminated with *salmonella* sp. and the highest count of $3.2\pm0.1x10^4$ CFU/g recorded with Egg roll sample and suya meat sample of $2.6\pm0.6x10^4$ CFU/g. *Salmonella* sp. are usually implicated in most foodborne diseases and even a small number of this pathogen in foods have the potential to cause severe illness (Health Protection Agency, 2009; Hull-Jackson *et al*., 2019). The presence of *Salmonella* spp. in foods may have been due to poor hygiene practices and cross-contamination. Such risky practices predispose food to recontamination with eventual devastating consequences on consumers' health.

Total coliform count ranges as observed from different foods sampled in the study were higher when compared with minimum standards of quality that the Department of Health, South Africa (2000) recommends for the fitness of foods for human consumption. Akindele and Ibrahim, (2016) described the presence of coliform bacteria in food samples as a result of faecal contamination and potential foodborne illnesses such as diarrhea, dysentery, etc.

Coliform bacteria isolated from the foods sampled pose a significant public health and food safety concern. It is possibly as a result of poor hygiene practices, inadequate cleaning, or equipment contamination which could lead to spread of illnesses. Coliforms are indicator organisms; their presence in ready-to-eat foods portends possible danger. Coliform counts of $\geq 10^6$ recorded for Moi moi, Meat pie, Egg roll and Suya meat calls for strict adherence to standard food practices and effective HACCP application.

The percentage frequency of biofilm and haemolysis production ability of the identified bacterial isolates recorded in this study reveals that different isolates are biofilm producers; and their percentage frequency for biofilm production is quite alarming. This is in line with the finding of Allam *et al.*, (2017), who recorded high percentage of biofilm producing bacteria from ready-to-eat foods.

The high percentage of biofilm production recorded in the study is quite alarming and worrisome. Such bacteria have the ability to form complex communities on surfaces and encased in a productive matrix. High percentage frequency of biofilm production is of public health significance because biofilms enhance bacterial survival and persistence, increase resistance to antibiotics and disinfectants, facilitate horizontal gene transfer and contribute to the development of chronic foodborne infections (Donlan, 2002).

Results of Haemolysis production ability of bacteria isolates also called for serious attention. Bacteria showing positive haemolytic ability tend to possess virulence properties, which could break down red blood cells releasing hemoglobin (Proctor, 2016). Haemolysis production is a virulence factor that indicates that the bacteria have the potential to cause disease. High haemolysis production is associated with increased virulence and the ability to cause severe infections, such as sepsis and meningitis (Lowy, 2003).

Bacterial isolates that showed high percentage frequency of biofilm production and haemolysis production are of public health and food safety concern because they indicate potential for chronic foodborne infections, antibiotic resistance and severe disease outcomes. These factors highlight the importance of appropriate infection control measures, antibiotic stewardship and prompt treatment to prevent the spread of these virulent bacteria strains (Donlan, 2002).

Staphylococcus aureus, *Bacillus cereus*, *Escherichia coli*, *Vibrio* sp, *Athrobacter* sp, *Shigella sonnei*, *Pseudomonas putida*, *Cronobacter* and *Klebsiella* spp were isolated from the ready-to-eat foods, indicating poor sanitary control and practices. These organisms are known food borne pathogens and opportunistic pathogens that have been implicated in food borne disease outbreaks (Mudgil *et al.*, 2004; Oranusi *et al.*, 2007). This study revealed that while some of the foods sold in the markets are contaminated beyond acceptable microbiological limits, most of the foods are of acceptable microbiological standard.

Staphylococcus sp had the highest bacterial prevalence and occurrence, followed by *Bacillus* sp, *Shigella* sp, *Escherichia* sp, *Pseudomonas* sp, *Cronobacter* sp, *Vibrio* sp, *Klebsiella* sp and *Arthrobacter* sp. This indicates high levels of bacterial contamination and their presence in RTE foods poses a significant risk to consumer health, particularly in vulnerable populations. This study is consistent with previous studies conducted in similar markets in Nigeria (Oluwafemi and Semisaye, 2013). This hazardous situation is an indication of contamination from the skin, mouth or nose of food vendors (Bereda *et al.*, 2016). According to Stefano and Marina (2018), *Staphylococcus aureus* in foods may secrete toxins that cause food poisoning, and its presence in foods should not be tolerated because of the possibility of widespread food poisoning it can cause. Studies also shown that presence of *Staphylococcus sp.* in RTE could be attributed to contamination during food processing and handling, poor hygiene practices among food handlers, inadequate temperature control during storage and transportation and lastly cross-contamination from other foods or surfaces ((Mazizi *et al*., 2017). Food manufactures and handlers should implement strict hygiene practices and temperature control measure to reduce the risk of *staphylococcus aureus* contamination.

Bacillus sp also recorded in this study is quite alarming compared with the range by WHO (2014) of $<10^5$ CFU/g recommended for foods to be fit for human consumption (Centre for Food Safety, 2014). *Bacillus* sp presence in RTE suggests poor handling practices and control (Oluwafemi and Semisaye, 2013). It could be as a result of spores deposited in raw materials like meat, spices, onion and pepper, etc. used during food processing (Ishaq *et al*., 2018). The isolation of *Bacillus* sp from ready-to-eat foods may mean that its heat-tolerant spores may have survived cooking even though the vegetative form gets eliminated (Nemo *et al*., 2017). Consumption of such contaminated foods may result in foodborne illnesses (Centre for Food Safety, 2014).

Escherichia coli in food samples indicates faecal contamination which might occur during preparation or from the material used. Faecal contamination of food is hazardous to the health of consumers owing to the notoriety those organisms (Oranusi *et al.*, 2007).

Vibrio spp are a group of bacteria that are commonly found in environments and can contaminate food, particularly ready to eat foods and seafood. Consumption of contaminated food could lead to foodborne illness, which could be severe and even life threatening in some cases and of public health (Oranusi *et al.*, 2007).

These pathogenic bacteria are known to cause foodborne illness, highlighting the potential health risk to consumers. Their presence in this study may be attributed to poor hygienic practices, faecal and cross-contamination during food preparation, inadequate food processing, inadequately cleaned utensils, poor storage, the source or quality of water used for their processing and temperature control. Hawking of these foods in an open market also predisposes them to dust particles which may harbour pathogens that lead to food poisoning upon consumption. They are at high risk of exposure to food-borne illnesses and pathogenic microorganisms could thrive in them and cause infection upon consumption. Studies have that these organisms are known food borne pathogens and opportunistic pathogens that have been implicated in food borne disease outbreaks (Mudgil *et al.*, 2004; Oranusi *et al.*, 2007; Tambeker *et al.*, 2008; Yadav *et al.*, 2011).

In conclusion, the study revealed that a significant proportion of ready-to-eat foods sold in Mile 3 Market, Port Harcourt, were contaminated with pathogenic bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Shigella*, *Pseudomonas* sp, *Cronobacter* sp, *Vibrio* sp, *Klebsiella* sp and *Arthrobacter* sp. The high level of bacterial contamination in the study poses a significant risk to consumer health, particularly in the vulnerable populations such as the elderly, pregnant women and immunocompromised individuals. The finding suggests that poor handling, storage and hygiene practices among food vendors, as well as inadequate regulatory oversight contribute to the microbiological contamination of ready-to-eat foods in the markets. Routine microbiological analysis of ready-to-eat foods sold in mile 3 market and other markets in Nigeria is paramount to curtail any disease outbreak in form of food poisoning due to these pathogens.

The risk of foodborne illness can be prevented by ensuring that food is handled and stored properly and that food vendors and consumers are aware of the risks associated with consuming contaminated food. Such practice if dutifully followed will ensure that quality foods are sold to unsuspecting customers, and the emergence and spread of foodborne outbreak through them will also be contained. Food handlers should also be educated and be observant of current public health guidelines in their profession so as to minimize food-borne related illnesses. Finally, there is need for regular monitoring of the quality of ready-to-eat foods sold in markets.

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