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Bacteriological Assessment of Hawked Ready-To-Eat Fried Prawns Retailed in Selected Locations of Lagos Mainland, Nigeria

Kester, C. T.*, Daramola, J. A. and Shado, M.O.
Department of Biological Sciences,
Bells University of Technology,
Ota, Nigeria.

Email: christieradeke@gmail.com

Abstract
Ready-to-eat (RTE) prawn product which characterizes many streets of Lagos Mainland undoubtedly exposes a large number of customers to bacteriological hazards of health risks but no ample report yet on the safety of this product in Lagos Mainland. This study examined the bacterial contaminants of health importance in the hawked RTE prawns samples. Ten nylon-wrapped RTE prawns each were randomly selected from Oshodi (OS), Ebute-metta (EM) and Ketu (KT) vending areas of Lagos Mainland and bacteriologically enumerated for Total Aerobic Bacterial Count (TABC), Total Staphylococcus count (TSC) and Total Coliform Count (TCC), all expressed in log CFU/g. Presence of Salmonella and Shigella was also determined and biochemically confirmed. The bacterial counts differed insignificantly ($p<0.05$) among the vending sites; lowest and highest mean TABC values (4.70±0.15, EM; 6.72 ± 0.18, KT), TSC (4.46±0.10, KT; 5.86± 0.13, OS), TCC (4.36±0.13 (OS); 5.55±0.06, (OS) and TEBC (0, (EM); 5.48±0.11, OS). Biochemically identified Staphylococcus aureus dominated the prawn products. Escherichia coli were detected with traces of Salmonella spp. The highest bacterial counts recorded in the entire RTE fried prawn products of this study are within the maximum limits recommended, implying their safe consumption. However, detection of Escherichia coli and Salmonella sp. is an object of public health concern which demands an improvement in the hygiene practices of the product handlers.

Keywords: Bacteriological safety, Hawked fried prawns, Lagos Mainland, Ready-to-eat

INTRODUCTION
Ready-to-eat (RTE) foods are consumed as snacks at the point of sale (Ibe *et al*., 2015). RTE foods such as prawns have become popular in the last two decades, particularly in cities (Choi, 2022). Prawns are a type of seafood that constitutes a very important fraction of a healthy diet rich in quality protein and other valuable nutrients that are low in saturated fatty acids but may contain omega-3 fatty acids (Kakara *et al*., 2016). Hawked RTE prawns have grown highly popular in major cities and towns in Nigeria especially Lagos State, serving as source of income for low income earners in urban areas and at the same time serves as source of food to both the rich and poor. Some people in urban areas feed on prawns almost in alternate days and many are oblivious of their health implications associated with sanitary conditions of their processing and poor packages.

Oranusi and Nubi (2016) claimed that the consumption of prawns as a healthy diet is not risk free but can be exposed to a range of hazards from catching them in water to the table as food. Some of these hazards, according to Iwamoto *et al.* (2010) can involve bacteria, natural toxins

*Author for Correspondence

and chemical contaminants which are introduced by humans and are responsible for important proportion of most food borne illnesses and outbreaks.

Species of bacteria associated with seafood borne illnesses include but not limited to species of *Vibrio, Salmonella, Shigella, Campylobacter, Listeria* and pathogenic *Escherichia coli*, *Cryptosporidium, Cyclospora cayetanensis, Giardia lamblia* (Ali *et al*., 2020). The growth of such pathogens is enhanced by the tropical climatic conditions.

Salmonella is a mesophilic pathogen associated with prawns reared in intensive aquaculture or found in waters contaminated with faeces. Species like *Campylobacter* and *Salmonella* are known to contaminate prawns by transfer from infected workers to prawns (Todd *et al*., 2008). *Staphylococcus aureus* is not a bacterium intrinsically associated with prawns except through processing by the workers. Healthy individuals carry toxin-producing strains as part of the natural skin microflora through transfer of bacteria to prawns during handling (Otto, 2014) which could pose a serious health challenge to consumers.

Food safety forms a critical part of public health challenge in Nigeria. The Federal Ministry of Health in Nigeria reported about 90,000 cases of food poisoning in 2007 while the World Health Organization (WHO) estimated 200,000 deaths from diarrhoea each year in Nigeria (WHO, 2006) through contaminated food and water. The Food and Agricultural Organization of the United Nations and the World Health Organization (FAO/WHO, 2000) stated that illness due to contaminated food is perhaps the most widespread health problem in the contemporary world and an important cause of reduced economic productivity (Newman *et al*,. 2015). However, there is scanty information on the safety of hawked RTE prawns on the health of the consumers especially in this study area of Lagos.

This study identified the species of bacteria in RTE prawns hawked in some selected locations in Lagos Mainland, Nigeria. Research into the bacteriological safety of hawked RTE prawn products is with a view to improving their quality, consumer safety and sanitary suggestions for the handlers.

MATERIALS AND METHODS

Area of study

Study samples (Plate 1) were purchased at Oshodi (6.5355ºN, 3.3087ºE), Ebute-metta (6.4817ºN, 3.3769ºE) and Ketu markets (6.5973ºN, 3.3904ºE) all located in Lagos Mainland.

Plate 1- Nylon-packed samples of fried RTE Prawns products vended in one of the study locations.

Samples collection and Preparation

Thirty (30) wraps of nylon packs, each having 10 pieces of RTE fried prawns, were purchased from The method of bacteriological analysis described by Harrigan and McCance, (2014) was adopted for the analyses of prawn samples. Each of the samples was pounded into pieces in a sterilised mortar and 10g was aseptically weighed into 90mls of sterilised normal saline diluent to make homogenate of 10^{-1} dilution. Standard decimal dilution was used to determine the bacteriological loads. The parameters determined were Total Aerobic Bacterial Count (TABC), Total Coliform Count (TCC), Total Staphylococcus Count (TSC) and Total Enterobacterial Count (TEBC) using Nutrient agar (NA), MacConkey agar (MCA), Mannitol Salt Agar (MSA) and Eosine Methylene Blue Agar (EMBA) respectively. Presence of *Escherichia coli* and *Salmonella spp.* was also tested on Salmonella-Shigella Agar (SSA) and Lactose Broth (LB).

Total Aerobic Bacterial Count (TABC) was determined using pour plate technique. 1ml of inoculum from the dilution of choice was dispensed in triplicates into labelled sterile Petri dishes. About 20ml of sterile molten nutrient agar medium, cooled to about 45°C, was poured into each of the inoculated Petri dishes, swirled gently to mix the molten agar thoroughly and uniformly with the inoculum and allowed to gel. The Petri dishes were inverted and incubated at 37°C for about 24 hours after which the Petri dishes containing 30-300 colonies per dilution were counted and recorded. Total counts were expressed as colony forming units per gram of sample ($CFUg^{-1}$). The isolates were purified, Gram stained and the cell morphologies microscopically examined.

The same isolation procedure in TABC was used to isolate *E.coli* colonies using EMBA and incubated at 37ºC for 24hrs. Green metallic sheen colonies were suspected as *E.coli*.
To detect presence of *Salmonella sp.* and *Shigella sp.*, 1 ml of the homogenized prawn sample was transferred to 10 ml of lactose broth for the pre-enrichment of *Salmonella spp*. and *Shigella spp.,* and incubated at 37ºC for about 6 hours. A loopful of the lactose broth was aseptically streaked on the *Salmonella Shigella* (SS) agar plates to confirm for the presence or absence of these organisms. The plates were incubated at 37ºC for 18 - 24 hours for the development of typical Salmonella colonial characteristics which should appear colourless with black centres' indicating production of hydrogen sulphide while Shigella do not and the colonies remain colourless.

Identification of Isolates (Biochemical Tests)

Colonies of bacterial isolates were purified on surface-dried Nutrient agar plates by streak plate technique of isolation. The plates were incubated at 37ºC for about 24 hours and the pure colonies were biochemically examined for colonial characteristics, Gram staining reaction and other tests including catalase production, indole, citrate utilization, coagulase, oxidase, urease production, sugars fermentation and motility by following standard protocol used by Alfred, (2007).

Statistical analysis

The experimental design applied was a completely randomized technique. Data were subjected to one-way ANOVA followed by Duncan Multiple Range test used to compare differences among individual means. All statistical analyses were performed using SPSS 17.0 (SPSS, Chicago, IL, USA).

An informal discussion on the handling of prawns by potential customers that purchase them from the hawkers was carried out to establish the likely sources of contamination of the product.

RESULTS

Bacterial counts of vended fried RTE prawns samples

The mean total aerobic bacterial count (TABC) values of fried RTE prawn samples from the three vending sites: Oshodi (OS), Ebute-metta (EM) and Ketu (KT) are shown in Figure 1 to differ significantly ($p<0.05$). The values are generally high, with the highest mean range of 5.77 ± 0.11 to 6.72±0.18 log $CFUg^{-1}$ recorded in KT site, closely followed by OS (5.62±0.09 to 6.71±0.09 log $CFUg^{-1}$) and the least of 4.70±0.15 to 6.65±0.12 log $CFUg^{-1}$ in EM.

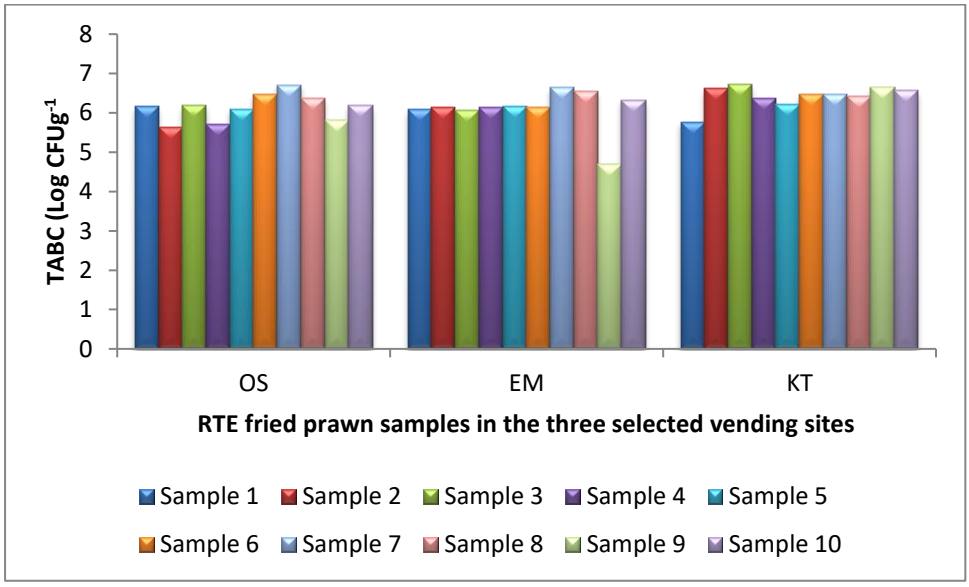

Figure 1: Total aerobic bacterial count of fried RTE prawn samples from the three selected markets

In Figure 2, the mean Total Staphylococcus count (TSC) displayed the value ranges of 4.74±0.08 to 5.86±0.13 (OS), 4.63±0.12 to 5.73±0.08 (EM) and 4.46±0.10 to 5.75±0.12 log CFU/g (KT). However, the mean Total coliform counts (TCC) presented in Figure 3 ranged between 4.36±0.13 and 5.55±0.06 in OS products, 4.42±0.13 and 5.34±0.16 (EM) while KT samples ranged from 4.53±0.09 to 5.35±0.11 log $CFUg^{-1}$.

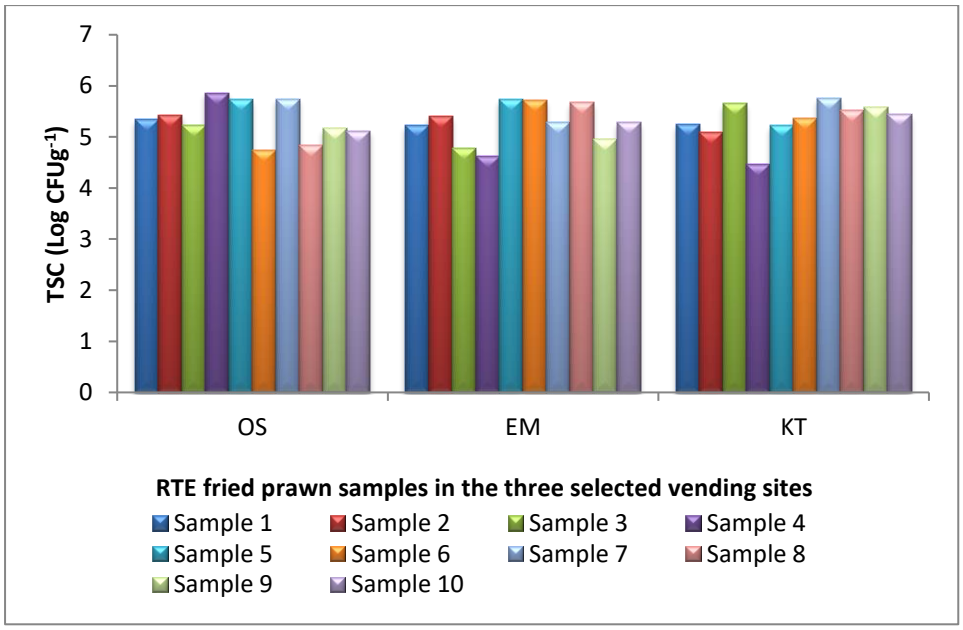

Figure 2: Total Staphylococcus count of fried RTE prawn samples from the three selected markets

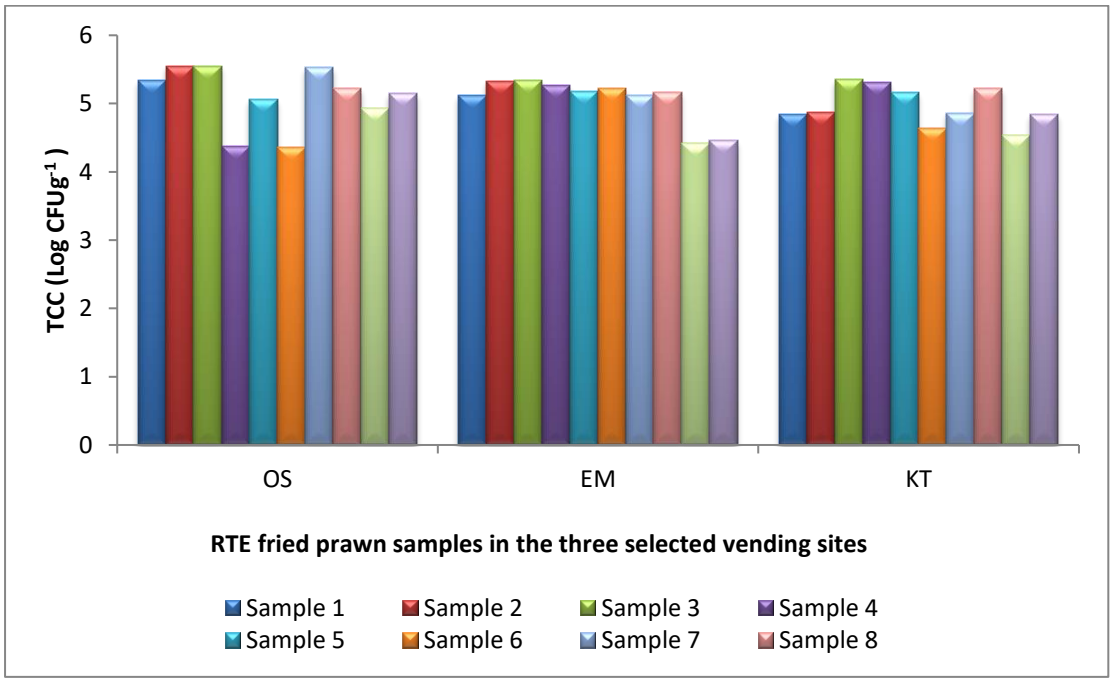

Figure 3: Total coliform count of fried RTE prawn samples from the three selected markets

Figure 4 showed variations in the non-detectable levels of enterobacteria in the sampled products of the three vending sites in the order of 20% (OS), 80% (EM) and 10% (KT) for which the highest mean values of 5.48±0.1, 4.32±0.15 and 5.42±0.11 log $CFUg^{-1}$ were respectively recorded.

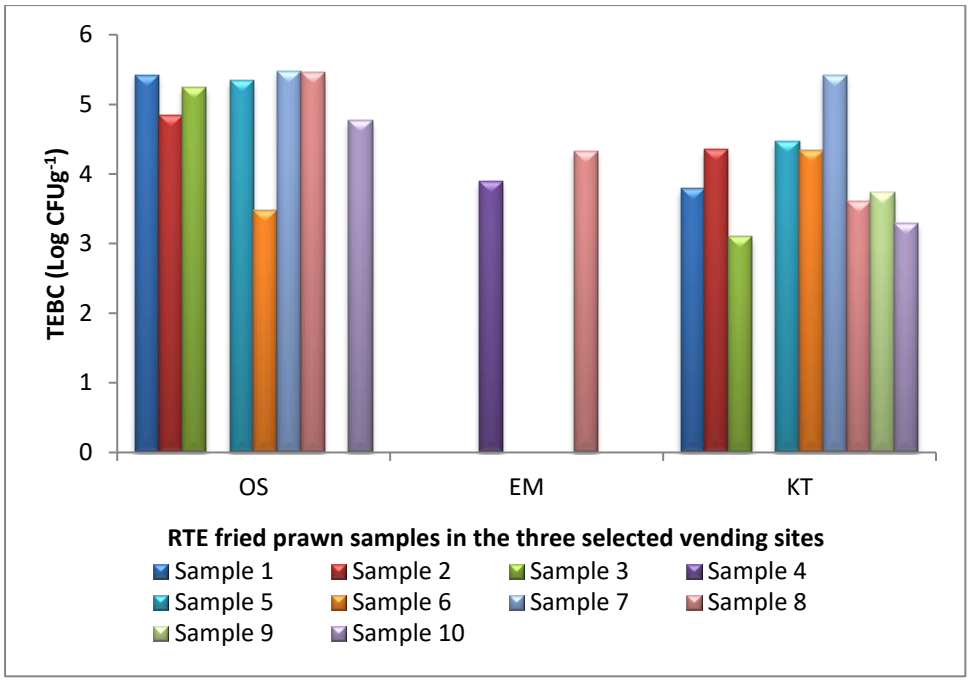

Figure 4: Total enterobacterial count of fried RTE prawn samples from the three selected markets

Majority of products sampled from EM site were, however, below detectable levels, with just 20% showing records of 3.90±0.06 and 4.32±0.15 log $CFUg^{-1}$.

The colonial, microscopic and biochemical characteristics of the probable bacterial isolates common to the fried RTE prawns are displayed in Table 1 and their percentage occurrence represented in the Figure 5.

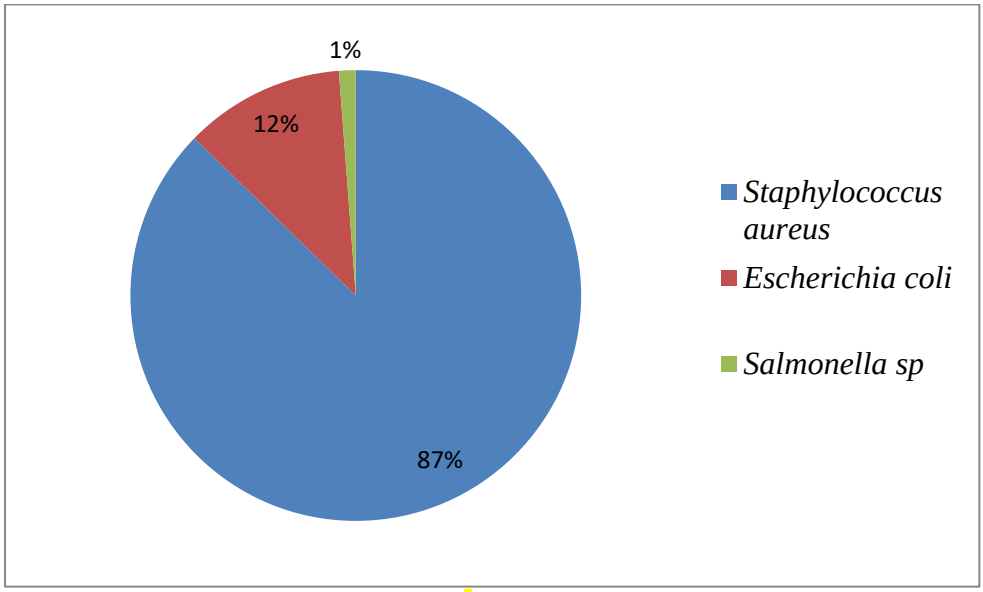

Figure 5: Percentage occurrence of bacterial isolated from fried RTE prawns products sampled from the selected markets

Identification of the bacterial isolates of the vended fried RTE prawn samples

Figure 5 shows a total of 469 bacterial isolates of the vended fried RTE prawn samples with the predominant Gram stained, microscopically examined and biochemically tested bacteria identified as *Staphylococcus* spp, *Escherichia coli* and *Salmonella* spp. (Table 1). The isolates occurred respectively in a descending order of 87.3% (409), 11.5% (54) and 1.2% (6).

Table 1: Colonial, microscopic and biochemical characteristics of bacterial isolates of fried ready-to-eat prawns

Colony Morphology	Gram's rxn	Microscopic examination	Ca	Co	Mo	G	SFM	SFG	IND	CIT	Ox	H_2S	U	Probable organisms
Flat, circular, golden yellow	+	Cocci in clusters	+	+	-	+	+	+	-	-	-	-	+	*Staphylococcus aureus*
Flat, green metallic sheen	-	Short rods	+	-	+	+	-	+	+	-	-	-	-	*Escherichia coli*
Transparent with dark centers	-	Rod shaped bacillus	+	-	+	+	+	+	-	-	-	+	-	*Salmonella* spp.

Ca – Catalase, Co – Coagulase, G - Gas Production, Ind – Indole, CIT – Citrate, H_2S – Hydrogen Sulphide
U – Urease SFG - Sugar Fermentation Glucose, SFM - Sugar Fermentation Maltose, Ox – Oxidase

DISCUSSION

The TABC values of the hawked Ready-to-eat (RTE) prawn products displayed in Figure 1 are significantly different ($p<0.05$) among the sampled vending sites of study. All sampled products ranged from 4.70±0.15 (Ebute-Metta) to 6.71±0.09 log CFU/g (Ketu) thereby ranking the products of good quality, based on the limit of Aerobic colony counts of 10^6 to $< 10^7$ declared by Microbiological Guidelines for ready-to-eat foods (2014). The bacterial count limits of 5 log 10 CFU/g (5.7 log 10 CFU/g) and 10^7 (7 log 10 CFU/g) recommended by the ICMSF (1986) respectively for good quality (m) and marginally acceptable quality products (M) for fish and fishery products equally rank the sampled products of this study of safe consumption status as at the period of study. It is, however, not impossible that the declared marginally acceptable bacterial loads of the RTE prawn products of this study could fall beyond acceptable limit to unsatisfactory microbial quality counts of $\geq 10^7$ with prolonged hawking period. This is because the increased population of the intrinsic bacterial flora is bound to be facilitated by the tropical climatic conditions. The consumption of such products at this point will, nevertheless, be detrimental to the ultimate consumers, even if the isolates are not pathogenic (Sudershan *et al.* 2012). On the contrary, however, majority of the values recorded in this study are higher than the Total Aerobic Plate Count range of 10^4 to 10^5 recorded by Oranusi and Nubi (2016) in their study on microbiological safety evaluation of RTE shrimps and snails sold along Lagos-Shagamu Express way. This can be attributed to the sources of the raw materials used as well as the variance in the hygienic and sanitary practices adopted by the vending locations. Although in some countries, zero (0) bacterial load in $25g^{-1}$ of sample is the acceptable microbiological level in cooked crustaceans (Oranusi *et al.*, 2016). The total bacterial counts of the fried RTE prawns of this study, as corroborated by Chukwu *et al.* (2013), could also possibly be contaminants from the prawns' flora that escaped postharvest frying or post-process contaminants from processing environment, water, utensils, and food personnel.

The TSC of the RTE fried prawns products sampled from Oshodi is shown in Figure 2 to be of good quality, values ranging from 4.46±0.10 to 5.86±0.13 log CFU/g. The occurrence of *Staphylococcus* spp. in of these study products is therefore suggestive of poor handling

practices exhibited by the vendors probably during packaging of the fried RTE prawns into nylon. This finding is similar to the work done by Oluwafemi and Simisaye (2009) who reported the presence of *S. aureus and Salmonella sp*. in sausages sold in Abeokuta and Benin City, Nigeria. According to their submissions, most of the sausages sold as RTE food pose health risk to consumers, making it imperative to institute not only sanitary measures during its production and sales bit for retailers selling raw of pre-processed foods to have a steady source of power supply. The presence of *S. aureus* in the RTE frozen foods in Ijora-Olopa, Lagos, studied by Okonko *et al.* (2008) of RTE frozen foods, is suggestive of contamination through handling by processors which might eventually affect the health of the consumers. Taylor *et al.* (2022) also attested that *S. aureus* are a part of the normal flora of human skin, hair and nose and their presence in food is an indication of poor hygiene during food processing and handling. Some strains of this isolate, however, produce heat-stable enterotoxins which can cause gastrointestinal disorder in consumers (Otto, 2014).

The TCC values ranging between 4.36±0.13 and 5.55±0.06 log CFU/g recorded in the products sampled from Oshodi site (Figure 3) are beyond the borderline limit of 10^2 - ≤ 10^4 recommended by Microbiological Guidelines for ready-to-eat foods, (2014). Such undesirable levels of coliforms suggested possibility of contamination most especially by animal faecal materials, poor sanitation practices and cross contamination during storage, handling, processing or pollution arising from poorly sealed container. According to Stratev *et al.* (2015), the presence of coliforms as indicator organisms could connote the possible presence of other enteric pathogens. In this study, vendors reportedly packed and arranged the products with bare hands in white cellophane wrappers which are poorly tied. The water for processing and utensils could also be sources of contamination. The hawkers of the study products reported that some customers do ignorantly open the tied cellophane wrappers to check the product with bare hands which may not eventually be purchased. The bacteriological quality integrity of such products must have been tampered with and undoubtedly constituted health risk to the potential consumers.

The TEBC values ranged from zero to 5.48±0.11 Log CFU/g respectively in Ebute metta and Oshodi vending sites as displayed in Figure 4. In Ebute metta, only 20% of the fried prawn samples were positive for the growth of Enterobacteria. The values ranged from zero to 3.90±0.06, 4.32±0.15 log $CFUg^{-1}$ being the highest. This record ranked Ebute metta products the best in enterobacterial quality, just 10% falling beyond the unsatisfactory level of ≥10^4 recommended by Microbiological Guidelines for ready-to-eat foods (2014). This observation can be accredited to the freshness of the fried RTE prawn products as a result of the proximity of the vending site to Makoko hamlet, a water-front settlement in the centre of Lagos, renowned for abundant supply of fresh raw and processed fishery products, shrimps and prawns inclusive. This is in agreement with the findings of Mustafa and Abdulla (2011), who reported the counts of 2.3 to 4.4 log $CFUg^{-1}$ in Sudanese street-vended traditional foods. Oshodi vending site, being a major market area in Lagos metropolis, recorded the highest value of 5.48±0.11 log CFU/g. This could be as a result of its comparative denser market population as well as its far distance to the major product depot.

Escherichia coli, Salmonella spp*, and Staphylococcus aureus* isolated from and identified in the fried RTE prawn products of this study are commonly associated with most RTE food samples and are reported by Okonko *et al.* (2008) as those commonly found in soil and water. The presence of *Salmonella* spp. in food items can imply post-harvest contamination, inadequate temperature control, infected food handlers, contaminated raw ingredients, inadequate heat treatment and cross contamination (Heinitz *et al.* 2000). This finding is similar to the work

done by Oluwafemi and Simisaye, (2005) who reported the presence of *S. aureus and Salmonella sp*. in sausages sold in Abeokuta and Benin City, Nigeria. According to them, most of the sausages sold as RTE food pose health risk to consumers. This suggests that aside making it imperative to establish sanitary measures during production and sales, retailers selling raw or pre-processed foods need steady source of power supply, a condition which may not be currently attainable in a developing country like Nigeria.

However, microbes of medical concern such as *Bacillus cereus*, *Enterobacter aerogenes, Flavobacterium* sp. and *Micrococcus* sp. encountered by Okonko *et al.* (2008) in their studies on RTE seafoods samples in Ijora-Olopa were not discovered in the prawn samples of this study. The non-detection of such microbes can be attributed to the frying processing to which the fried RTE prawn products of this study were subjected.

CONCLUSION

The bacterial loads of all the fried RTE prawn products of this study were within the maximum limit recommended for fishery products of acceptable quality, the elevation of which is definite with storage of the sales left over as well as handling by customers. The detection of enteric organisms (*Escherichia coli and Salmonella* spp.) indicated that the consumers may be at risk of contracting food borne infections possibly attributed to poor hygiene during handling. The consumption of fried RTE prawns cannot be banned but measures can be taken to educate the vendors about safety and hygienic practices.

The microbiological safety of food is achieved as far as possible by ensuring the absence of pathogenic microorganisms and by all means preventing their multiplication. In order to reduce the initial bacterial load of the prawn products to the barest minimum, the handling techniques involved in the processing of the product calls for improvement. Realization of this lies in the adoption of one of the concepts used to identify microbiological vulnerable points in the food production process and processing. Hazard Analysis Critical Control Point (HACCP) concept usually encompasses such methods as improved handling techniques, monitoring of temperature and more intensive supervision.

The success of the impartation of the HACCP concept on the food handlers and vendors can be done on a pilot scale to assess the level of adaptability. In order to prevent the irrational opening of the prawn products by customers before buying, the processors can be encouraged to employ the use of simple machine to skilfully seal the packaging cellophane wrappers.

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