

Evaluation of Bacterial Load and Occurrence of *enterotoxigenic coagulase* Positive *Staphylococcus aureus* in ready-to-eat foods sold in Benin City

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Abstract: Ready-to-eat foods are easily recontaminated by microbes during post processing and may be the cause of food borne diseases. Food samples analyzed were Doughnut, Fried snail, Boiled yam/stew, Smoked fish, Moi-moi, Jollof rice, Eba and Gala obtained from vendors in Egor, Ikpoba Okha, Ovia North-East and Oredo Local Government Area (LGA) all in Benin City. The collected ready-to-eat foods were taken to the laboratory and analyzed immediately as 0 h and the other part was stored in a cool box for 8 hr. Pour-plated method was used for heterotrophic bacterial and Staphylococcal count on nutrient agar and mannitol salt agar respectively. Polymerase chain reaction assay was employed for the detection of *femA* and Staphylococcal enterotoxin (A, B, C, D and E) genes. The heterotrophic bacterial and Staphylococcal counts of the food samples increased after 8hr of storage. The highest bacterial count of $10.30 \pm 0.05 \times 10^6$ cfu/ml was from doughnut obtained from Ikpoba Okha LGA and the least was $1.90 \pm 0.10 \times 10^6$ cfu/ml from fried snail obtained from Egor LGA. The highest Staphylococcal count was $4.00 \pm 0.20 \times 10^6$ cfu/ml from doughnut obtained from Ikpoba Okha LGA and the least was $0.75 \pm 0.05 \times 10^6$ cfu/ml from fried snail obtained from Ovia N/E LGA. The identified bacterial isolates were *Bacillus cereus*, *B. subtilis*, *Corynebacterium kusteri*, *C. xerosis*, *Escherichia coli*, *Micrococcus varians*, *Staphylococcus aureus*, *S. epidermidis*, and *S. intermedium*. *Micrococcus varians* had the highest frequency of occurrence (11.75%). All the *S. aureus* isolated from the food samples obtained from the four LGAs harboured 100% *femA* and 75% SEE genes. The study showed that ready-to-eat foods are prone to serious contamination by potentially hazardous organisms whose population and toxigenicity increases with time.

Keywords: Foods, *Staphylococcus aureus*, enterotoxin, bacteria, PCR.

INTRODUCTION

Food is any substance which is likely of plant or animal origin and contains essential nutrients such as carbohydrate, proteins, vitamins and mineral consumed by an animal or human to provide nutritional support (Rawat, 2015). Ready-to-eat food is the food intended for direct human consumption without the need for cooking (Kotzekidou, 2013). These foods are not sterile because they contain microorganisms which can lead to food intoxication or infections when present above the acceptable limits (Okonko *et al.*, 2009). These foods are easily contaminated by microorganisms found in soil, air and food handlers' microflora (Kotzekidou, 2013; Kharel *et al.*, 2016). There are many of these causative microorganisms such as *Corynebacterium* spp, *Bacillus* spp, *Escherichia coli*, *Micrococcus varians*, *Streptococcus pyogenes* and *Staphylococcus* species are of public health significance (Esen and Owusu, 2013).

Therefore improper processing of food for consumption can result to food spoilage or food borne diseases. Food spoilage is mainly caused by invasion of mould, yeast and bacteria. Bacterial contamination is more dangerous because very often the food does not look bad even when severely infected, it may appear quite normal (Rawat, 2015). This can be prevented by Food rotation system, addition of preservatives, refrigeration and canning (Adesiyun, 1995).

Food borne diseases caused by microbiological agents are major problems faced by developing countries such as Nigeria (Madueke *et al.*, 2014). Common among the bacterial contaminants is *Staphylococcus aureus* responsible for a significant number of food borne diseases (Talaro and Talaro, 1996). *Staphylococcus aureus* is considered the third most important cause of foodborne diseases reported worldwide (Esen and Owusu, 2013). It is a leading cause of food poisoning which results from consumption of food contaminated with Staphylococcal enterotoxins. These enterotoxins (SEA-SEJ) are highly thermostable hence, normal cooking and pasteurization cannot totally deactivate them (Yan *et al.*, 2012).

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According to Saadati *et al.* (2011) 95% of Staphylococcal food poisoning outbreaks were caused by Staphylococcal enterotoxins (SEA-SEE) and the remaining 5% were associated with other Staphylococcal enterotoxins. The findings of CDC revealed that Staphylococcal food poisoning is ranked among the top five pathogens in the United States affecting 241,148 persons annually (Hait *et al.*, 2014). Several authors have reported that in Nigeria, *Staphylococcus aureus* is one of the most isolated bacteria that cause food-borne disease according to (Agbo *et al.*, 2016; Owuna *et al.*, 2015 and Ochei *et al.*, 2014). Food borne microbiological hazards may be responsible for as many cases of illness each year and are thus an important food safety challenge. To lower the incidence of food borne disease, many experts and stakeholders urge the development of science and risk-based food safety system in which decision makers prioritize hazards, interventions and reduction of risks (Kawo and Abdulmumin, 2009). The aim of this study was to evaluate the bacterial load and occurrence of enterotoxigenic coagulase positive *Staphylococcus aureus* in ready-to-eat foods sold in Benin City.

MATERIALS AND METHODS

Sample collection

A total of 32 food samples were collected from four local government areas (LGAs) Oredo, Egor, Ovia North-East and Ikpoba Okha all in Benin City Edo state. Food samples collected in duplicate were Smoked fish, Jollof rice, Eba, Fried snail, Boiled yam and stew, Doughnut, Moi-moi and Gala. Samples were obtained in surface sterilized flasks and taken to the laboratory within the hour of collection. The collected samples were shared into two parts, one was analyzed immediately they got to the laboratory as 0 h and the other part in the sterile flasks were kept in a cool box in the Microbiological laboratory and analyzed after 8 h of storage.

Bacteria enumeration, characterization and identification of food samples

From the food samples 1g was weighed and homogenized into 9 ml of sterile distilled water. From the homogenates serial dilution (ten-fold) using sterile distilled water was carried out. Aliquots of 1ml of the ten-fold dilutions above were pour-plated on nutrient agar and mannitol

salt agar for total bacterial and Staphylococcal counts respectively. Plates were incubated at 28 ± 2 °C for 24 h, after which discrete colonies were counted according to Cheesebrough, (2004).

Identification was carried out using cultural, morphological and biochemical tests. *Staphylococcus* sp. which showed characteristic white to deep yellow colonies on Mannitol salt agar plates were further screened for coagulase activity (Benson, 2002; Cheesebrough, 2004; Kerouanton, 2007).

Molecular analysis

A single colony of *Staphylococcus aureus* from the four LGAs was cultured in 5 ml nutrient broth on a shaker for 24 h at 28°C. DNA of *Staphylococcus aureus* was extracted from overnight grown pure isolates by transferring 1.5ml of cultures into 2 ml eppendorf tube and centrifuged at 4600 g for 5 mins. The cells pellets were washed twice with sterile distilled water. The resulting pellets were re-suspended in 520 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), after which 15 µl of 20 % SDS and 3 µl of Proteinase K (20mg/ml) were added. The mixture was incubated for 1 h at 37 °C, then 100 µl of 5 M NaCl and 80 µl of a 10 % CTAB solution in 0.7 M NaCl were added and vortexed. The suspension was incubated for 10mins at 65 °C and kept on ice for 15mins. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5mins and centrifugation at 7200 g for 20 mins. The aqueous phase was then transferred to a new tube and isopropanol (1:0.6) was added and DNA precipitated at -20 °C for 16 h. DNA was collected by centrifugation at 1300g for 10mins, washed with 500 µl of 70% ethanol, air-dried at room temperature for approximately three hours and finally dissolved in 50µl of TE buffer (Zschock *et al.*, 2000). The *Staphylococcus* enterotoxin genes were amplified using PCR which was carried out in a GeneAmp 9700 PCR System Thermal cycler with the following thermal cycling profile: an initial denaturation at 94 °C for 5 mins was followed by 35 cycles of amplification (denaturation at 94 °C for 2 mins, annealing at 57 °C for 2 mins, and extension at 72 °C for 1 min), ending with a final extension at 72 °C for 7 mins (Johnson *et al.*, 1991). The amplification of genes was carried out with a pair of primer documented by Mathenge *et al.* (2015) as shown in Table 1.

Gel electrophoresis was carried out with agarose gel of 1 % (w/v) which was allowed to solidify then stained with ethidium bromide. The molten agarose was poured into the casting tray, allowed to solidify and 1XTAE buffer was poured also to submerge the gel. Each PCR product was loaded after the 1000 bp DNA ladder which was loaded into wells respectively. The gel was

electrophoresed at 120 V for 45 mins, visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel (Zschock *et al.*, 2000).

Table 1. PCR primers and fragment lengths of the studied genes

Target gene	Primer	Oligonucleotide sequence (5' –3')	Size (bp)
SEA	F	GGTTATCAATGTGCGGGTGG	102
	R	CGGCACTTTTTTCTCTTCGG	
SEB	F	GTATGGTGGTGTAACTGAGC	164
	R	CCAAATAGTGACGAGTTAGG	
SEC	F	AGATGAAGTAGTTGATGTGTATGG	451
	R	CACACTTTTAGAATCAACCG	
SED	F	CCAATAATAGGAGAAAATAAAAG	278
	R	ATTGGTATTTTTTTTCGTC	
SEE	F	AGGTTTTTTTCACAGGTCATCC	209
	R	CTTTTTTTTCTTCGGTCAATC	
<i>FemA</i>	F	AAAAAAGCACATAACAAGCG	132
	R	GATAAAGAAGAAACCAGCAG	

STATISTICAL ANALYSIS

Descriptive statistics and Analysis of variance (ANOVA) was done using SPSS version 20. Scatter graph of correlation coefficient was carried out using Microsoft excel version 2007.

RESULTS

Total heterotrophic bacterial count of food samples from the four local government (Ikpoba okha, Ovia north/east, Egor and Oredo) is shown in Table 1. The bacterial count increased as the time increases and the highest bacterial count was observed after 8 h. After 8 h of storage, the food sample with the highest bacterial count was doughnut obtained from Ikpoba Okha LGA while the least fried snail obtained from Egor LGA with values 10.30 ± 0.05 and $1.90 \pm 0.10 \times 10^6$ cfu/g respectively.

Total Staphylococcal count of food samples from the four local government area (Ikpoba okha, Ovia north/east, Egor and Oredo) is shown in Table 2. The Staphylococcal count increased as the time increases and the highest Staphylococcal count was observed after 8h. After 8h of storage, the food sample with the highest Staphylococcal count was doughnut obtained from Ikpoba Okha LGA

while the least was fried snail obtained from Ovia N/E LGA with values 4.00 ± 0.20 and $0.75 \pm 0.05 \times 10^6$ cfu/g respectively.

Figure 1 represents the correlation coefficient (R^2) of Staphylococcal count and total heterotrophic bacteria count of the four local government areas sampled. There was a strong R^2 of Staphylococcal count and heterotrophic bacteria count at 8 h in all the samples obtained from the local governments with the values 0.99, 0.999, 0.996 and 0.998 respectively.

Table 3 showed the bacterial isolates and their frequency of occurrence (%). Nine (09) species of bacteria were isolated from the food samples. The isolates were identified as *B. cereus*, *B. subtilis*, *C. kustcheri*, *C. xerosis*, *E. coli*, *M. varians*, *S. aureus*, *S. epidermidis* and *S. intermedius*. The isolates with the highest frequency of occurrence was *M. varians* while the least was *E. coli* with percentage 11.75 and 9.60 % respectively. Gel electrophoresis confirming the presence of Staphylococcal toxin genes from the four LGAs are shown in Figure 2. Table 4 represents the toxin producing genes detected in *S. aureus* isolates from the four local government areas.

It shows that in the food samples obtained from the four LGAs SEE (25 %). SEB and SEC were not detected. had the highest frequency (75 %) followed by SEA (25 %) and SED

Table 2: Total heterotrophic bacteria count ($\times 10^6$ cfu/g) of ready-to-eat food at time intervals (0 and 8 h) from four LGAs of Benin City

Ready-to-eat food	Ikpoba Okha		Ovia North-East		Egor		Oredo	
	0	8	0	8	0	8	0	8
Fried snail	3.30 \pm 0.30	7.20 \pm 0.20	1.10 \pm 0.10	2.00 \pm 0.10	1.35 \pm 0.15	1.90 \pm 0.10	1.35 \pm 0.15	3.25 \pm 0.15
Doughnut	2.30 \pm 0.20	10.30 \pm 0.50	4.5 \pm 0.40	4.80 \pm 0.20	1.45 \pm 0.15	6.70 \pm 0.30	1.75 \pm 0.15	2.40 \pm 0.10
Smoked fish	3.85 \pm 0.35	4.80 \pm 0.20	4.2 \pm 0.40	6.20 \pm 0.30	4.05 \pm 0.35	4.20 \pm 0.20	2.85 \pm 0.25	4.20 \pm 0.20
Gala	2.15 \pm 0.25	3.65 \pm 0.15	3.95 \pm 0.45	3.05 \pm 0.15	1.25 \pm 0.15	2.10 \pm 0.10	2.55 \pm 0.25	3.90 \pm 0.10
Jollof rice	1.85 \pm 0.15	4.40 \pm 0.20	1.70 \pm 0.20	5.15 \pm 0.25	1.30 \pm 0.10	3.55 \pm 0.15	1.65 \pm 0.15	3.65 \pm 0.15
Boiled yam/stew	2.55 \pm 0.25	4.30 \pm 0.20	1.60 \pm 0.20	6.20 \pm 0.30	4.50 \pm 0.40	6.05 \pm 0.25	3.80 \pm 0.40	7.45 \pm 0.35
Moi-moi	2.05 \pm 0.25	3.15 \pm 0.15	1.60 \pm 0.20	3.75 \pm 0.25	2.80 \pm 0.30	3.25 \pm 0.25	2.20 \pm 0.20	5.45 \pm 0.25
Eba	2.85 \pm 0.25	2.90 \pm 0.10	4.85 \pm 0.45	7.70 \pm 0.40	1.10 \pm 0.10	2.40 \pm 0.10	2.45 \pm 0.25	4.20 \pm 0.20

Table 3: Staphylococcal count ($\times 10^6$ cfu/g) of ready-to-eat food at time intervals (hr) from four LGAs of Benin City

Ready-to-eat food	Ikpoba Okha		Ovia North-East		Egor		Oredo	
	0	8	0	8	0	8	0	8
Fried snail	1.30 \pm 0.10	2.80 \pm 0.10	0.45 \pm 0.05	0.75 \pm 0.05	0.55 \pm 0.05	0.75 \pm 0.05	0.55 \pm 0.05	1.25 \pm 0.05
Doughnut	0.90 \pm 0.10	4.00 \pm 0.20	1.75 \pm 0.15	1.90 \pm 0.10	0.55 \pm 0.05	2.60 \pm 0.10	0.65 \pm 0.05	0.95 \pm 0.05
Smoked fish	1.50 \pm 0.10	1.90 \pm 0.10	1.65 \pm 0.15	2.40 \pm 0.10	1.55 \pm 0.15	1.60 \pm 0.10	1.10 \pm 0.10	1.60 \pm 0.10
Gala	0.80 \pm 0.10	1.45 \pm 0.05	0.55 \pm 0.05	1.15 \pm 0.05	0.45 \pm 0.05	0.85 \pm 0.05	1.00 \pm 0.10	1.55 \pm 0.05
Jollof rice	0.75 \pm 0.05	1.70 \pm 0.10	0.65 \pm 0.05	2.00 \pm 0.10	0.55 \pm 0.05	1.35 \pm 0.05	0.65 \pm 0.05	1.45 \pm 0.05
Boiled yam/stew	1.00 \pm 0.10	1.70 \pm 0.10	0.65 \pm 0.05	2.40 \pm 0.10	1.75 \pm 0.15	2.35 \pm 0.15	1.45 \pm 0.15	2.90 \pm 0.10
Moi-moi	0.65 \pm 0.05	1.25 \pm 0.05	0.60 \pm 0.10	1.45 \pm 0.05	1.10 \pm 0.10	1.15 \pm 0.05	0.85 \pm 0.05	2.10 \pm 0.10
Eba	1.10 \pm 0.10	1.15 \pm 0.05	1.90 \pm 0.20	3.00 \pm 0.10	0.95 \pm 0.55	0.95 \pm 0.05	0.95 \pm 0.15	1.60 \pm 0.10

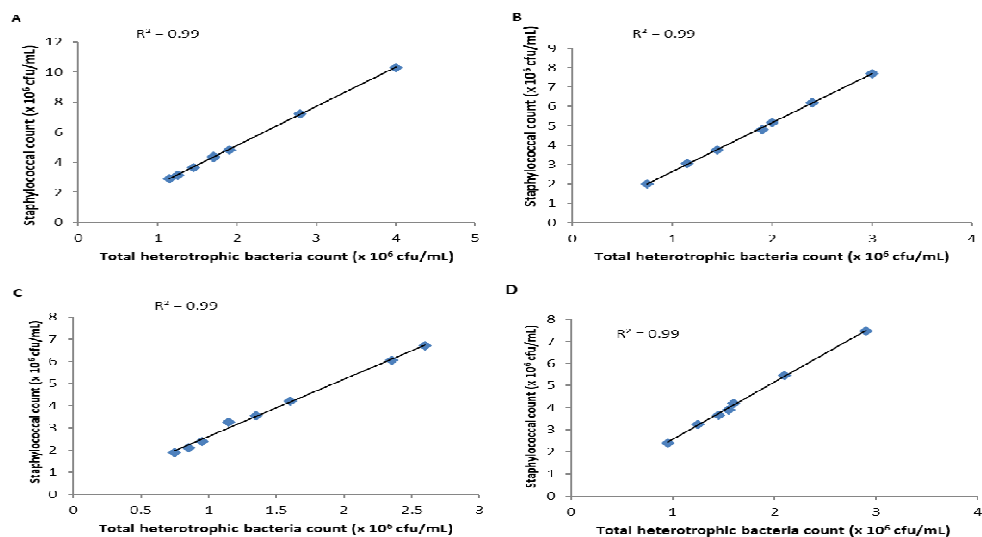


Figure 1: Correlation of Staphylococcal count and total heterotrophic bacteria count of the different LGA at 8hrs. A: Ikpoba Okha, B: Ovia North-East, C: Egor and D: Oredo.

Table 3: Frequency (%) Distribution of Bacterial Isolates

Bacteria Isolate	Frequency (%)
<i>Bacillus cereus</i>	10.95
<i>Bacillus subtilis</i>	11.05
<i>Corynebacterium kusteri</i>	10.48
<i>Corynebacterium xerosis</i>	11.45
<i>Escherichia coli</i>	9.60
<i>Micrococcus varians</i>	11.75
<i>Staphylococcus aureus</i>	10.92
<i>Staphylococcus epidermidis</i>	10.13
<i>Staphylococcus intermedius</i>	10.67

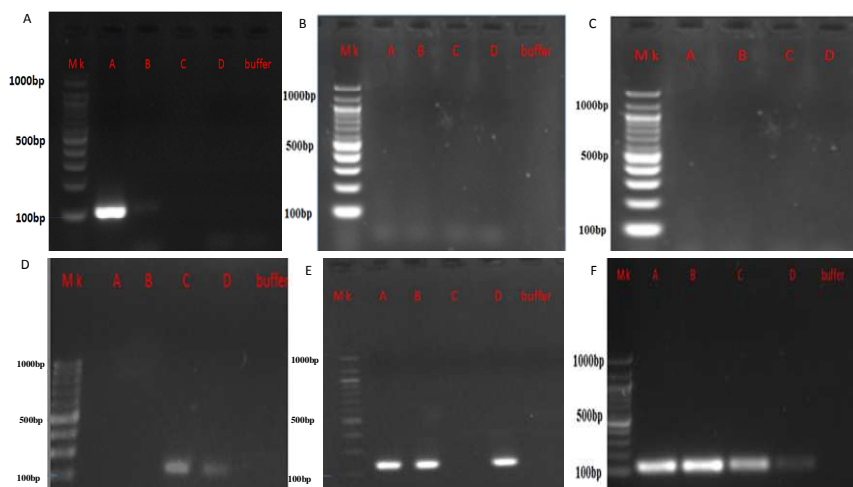


Figure 2: Electrophoresis of PCR products of *Staphylococcus aureus* toxin gene amplification A (SEA), B (SEB), C (SEC), D (SED), E (SEE) and F (*femA*). Isolates (Lane Mk: 1000 bp ladder; Lane A: Ikpoba Okha LGA Lanes B: Ovia N/E LGA Lane C: Egor LGA and Lane D: Oredo LGA)

Table 4: Frequency of Toxin producing genes detected in *S. aureus* obtained from all the LGAs

Toxigenic genes	% frequency
SEA	25
SEB	0
SEC	0
SED	25
SEE	75
<i>femA</i>	100

DISCUSSION

This study focused on ready-to-eat foods sold within four LGAs (Ikpoba Okha, Egor, Ovia North/East and Oredo) in Benin City Edo State. In the course of the study, eight different foods sold and consumed on the street were sampled for bacterial contamination

Bacterial guideline for ready-to-eat food stated that plate count must be $< 10^6$ cfu/g and staphylococcal count $< 10^4$ cfu/g (Centre for food Safety, 2007). The bacterial load of the food samples were higher than the acceptable limit, therefore their presence constitute a health challenges (Oranusi *et al.*, 2013; Madueke *et al.*, 2014). The high bacteria load (above the acceptable limit) obtained in all the sampled food items are indicative of post-treatment contamination in the light of the amount of heating that goes into food production (Ogugbue *et al.*, 2011). This can occur during cooling and exposure to the air which has been identified as the main source of microbial contamination of most ready-to-eat foods (Madueke *et al.*, 2014). Such a high viable bacterial count suggests practice of inadequate hygienic measures, mishandling and unhygienic condition of the retail shops in the four different locations. Jollof rice and Eba had the highest heterotrophic bacterial and Staphylococcal counts. Both ready-to-eat foods are starchy foods rich in sugars to support microbial growth but despite that, the two are packaged with materials that are not as cleans as supposed. Rice is wrapped in leaves of some local plant while Eba is packaged in small transparent nylon bags. These packaging materials could be a source of microbial contamination (Owuna *et al.*, 2015). Boiled yam/stew, Moi-moi and Doughnut also had high heterotrophic bacterial and Staphylococcal counts which were lesser than those of Jollof rice and Eba. Considering the preparation procedure of these foods, they involve a lot of handling during

and after preparations. All these contribute to the increased chances of microbial contamination of these food items. Fried snail and Smoked fish which were the only meat products sampled also had high heterotrophic bacterial counts. However their Staphylococcal counts were peculiarly higher than the other food items sampled. In China, raw meat, milk and dairy products, frozen products and cooked foods have been found as the major food types contaminated by *Staphylococcus aureus*, taking up 38%, 20%, 16% and 14%, respectively (Xu *et al.*, 2011). Also an investigation carried out in Calabar, Nigeria reported that incidence *Staphylococcus aureus* in foods such as fried yam. Moi-moi, suya and meat pie was 50, 83.3, 66 and 83.3 % respectively (Agbo *et al.*, 2016). The heterotrophic bacterial and Staphylococcal counts of the food items also increased with time

The implicated organisms were *Bacillus cereus*, *Bacillus subtilis*, *Corynebacterium xerosis*, *Corynebacterium kusteri*, *Escherichia coli*, *Micrococcus varians*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus intermedius*. Several authors had reported that ready-to-eat food harbor different agents of bacteria at varying load and could serve as good substrate for the microbial metabolism (Mensah *et al.*, 2002; Akinyemi *et al.*, 2013). The presence of *E. coli* in the food samples might be a source of concern as the bacterium is an indicator of faecal contaminant (Akinyemi *et al.*, 2013). The presence of *Bacillus* spp (*B. cereus* and *B. subtilis*) may be possible due to the presence of spores in the raw materials that may have survived cooking (Kharel *et al.*, 2016).

It is not surprising that a range of bacteria species were isolated from the street foods. A visit to the locations where these foods are hawked or sold offers enough conviction.

The stalls are quite unclean and are located on busy streets and highways where wind and traffic introduce lots of impurities into the food. The foods are displayed in open air or dirty show cases and most times are dished or wrapped with dirty plates washed with ground waters (Ayoade *et al.*, 2017). Most of the food handlers do not wear appropriate/protective catering clothes such as aprons, head covers, facial masks, gloves and the overall catering and hygiene practices of the vendors are generally below professional standards. Wogu *et al.*, (2011) reported that most proprietors of ready-to-eat food centers in Benin and probably elsewhere in Nigeria are not duly licensed, and their Staff are not properly selected. This situation is not peculiar to Edo state or Nigeria alone, several studies have revealed the frequent contamination of ready-to-eat food in many developing countries of the world. Studies by Rath and Patra, (2012); Suneetha *et al.*, (2011); and Arijit *et al.*, (2010) have revealed high loads of bacterial pathogens on popular ready-to-eat foods sold in different part of India. Mensah, *et al.*, (2002) reported that *Bacillus cereus*, *Staphylococcus aureus*, *Shigella sonnei*, *Escherichia coli*, and *Salmonella arizonae* were present in ready-to-eat foods sold in Accra, Ghana. Similar isolates as obtained in this study were also isolated from ready-to-eat foods; meat pie, beef sausage roll and egg roll, peeled orange, walnut and apple vended on highways; Onitsha-Owerri, South east, Nigeria (Oranusi and Braide, 2012). The findings are also in agreement with the works of Ajao and Atere (2009); Falola *et al.*, (2011); Mbah *et al.*, (2012); Ossai, (2012) and Madueke *et al.*, (2014) where diverse species of bacteria including: *Salmonella* spp., *S. aureus*, *E. coli*, *B. cereus*, *Shigella* spp., *Enterococci*, *Pseudomonas* spp., *Streptococcus* spp., *Flavobacterium* spp., *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Micrococcus* spp. and *Enterobacter* spp. were isolated from ready-to-eat foods sold in different parts of Nigeria.

In many countries of the world, *Staphylococcus aureus*, a Gram positive opportunistic pathogen can lead to a number of diseases ranging from skin lesions to septicemia or meningitis (Nester *et al.*, 2004). Some species of *Staphylococcus aureus* are known to produce Staphylococcal enterotoxins

(SE) in food resulting to food poison (Balaban and Rasooly, 2000; Wu *et al.*, 2016). These toxins are formed during *S. aureus* growth in foods (Cencil *et al.*, 2003). Foodborne illness associated with Staphylococcal enterotoxins commonly results in self-limiting symptoms including nausea, abdominal cramping, vomiting, and diarrhea (Biswajit *et al.*, 2008). Although Staphylococcal enterotoxins are predominantly produced by coagulase positive *S. aureus*, other Staphylococcal species both coagulase positive and negative have proven to be enterotoxigenic (Hait *et al.*, 2014). Typically considered a veterinary pathogen, *S. intermedius* have also been implicated in a food poisoning outbreak (Hait *et al.*, 2014). There are classical Staphylococcal enterotoxin (SE) serotypes: *SEA*, *SEB*, *SEC*, *SED*, and *SEE* and the more recently described *SEG*, *SEH* and *SEI*; all exhibit emetic activity (Hait *et al.*, 2014). Our investigation showed that *SEE* was the most common enterotoxin gene as three out of the four isolates had *SEE* gene. Other studies involving enterotoxin genotype analyses (Christiane *et al.*, 2001; Chen *et al.*, 2004) have previously reported the possibility of *S. aureus* strains possessing more than one enterotoxin genes. One of the four isolates had genes for both *SEA* and *SEE* while the last had gene for *SED* toxin only.

The result were at variance with the findings of Balaban and Rasooly, (2000) where *SEA* was reported as the most common enterotoxin found during food and food poisoning outbreaks worldwide, including Korea. They also reported linking specific enterotoxin genes to certain different origins which could be animals or food stuffs. Oh *et al.* (2007) reported that 50% of the poultry strains they studied carried genes for enterotoxin D production. Hence, the production of enterotoxin D may be characteristic of enterotoxigenic strains originating from poultry (Sharma *et al.*, 2000). Enterotoxin C was most commonly associated with cows having mastitis and with dairy products (Sharma *et al.*, 2000), and *SEA* was prevalent in livestock products (Su and Wong, 1997). In this study, however, a prevalent enterotoxin type linked to a specific food was not found. Perhaps this was because the foods investigated in this study were not specific foods but, rather, mostly multi-ingredient foods.

All the food types sampled in this study had Staphylococcal counts $> 10^6$. Generally, growth of *S. aureus* is necessary for enterotoxin production, although enterotoxin production does not always accompany growth, and in a few cases toxin production has been observed in non-replicating cell cultures, most recently by Wallin-Carlquist *et al.* (2001) in ham products.

CONCLUSION

The bacterial load of the ready-to-eat foods in this study was higher than the stipulated

microbiological limits, hence their presence constituted a health risk. This study also created an awareness on the genotypes of Staphylococcal enterotoxins present in the ready-to-eat foods hawked in the sampled areas; this is a useful database for epidemiological purposes. This can be adjudged that the street food retailed in most locations at Ikpoba Okha, Egor, Ovia North/East and Oredo LGAs of Benin City Edo state, as obtained in this study are not bacteriologically fit for consumption. Hence care should be taken to avoid a case of food poisoning.

REFERENCES

- Adesiyun A.A. (1995). Bacteriologic quality of some Trinidadian ready to consume foods and Drinks and possible health risks to consumers. *Journal of Food Protection* **58**(3): 51-655.
- Agbo, B.E., Udoekong, N.S. and Ozumba, R.E. (2016). Incidence of *Staphylococcus aureus* in street vended food sold in Calabar municipality, Nigeria. *Journal of Biopesticides and Agriculture* **3**(1): 31 – 40.
- Ajao, A.T., and Atere, T.G. (2009). Bacteriological Assessment and Hygienic Standard of Food Canteens in Kwara State Polytechnic, Ilorin, Nigeria. *African Scientist Journal* **3** (10): 173-180.
- Akinyemi, K., Fashola, M., Habib, N. and Akinwande, E. (2013). Vended Foods In Lagos, Nigeria: A potential reservoir for the spread of emerging strains of drug resistant bacteria. *Health* **5**(4):675-80.
- Arijit, D.G.S., Nagannada, S.B. and Shilpi, B. (2010). Microbiological Quality of Street-Vended Indian Chaat sold in Bangalore. *Journal of Biological Sciences*. **10** (3): 255 -260.
- Ayoade, A.A., Gbolahan, S.S. and Ayodele. S. R. (2017). Assessment of Road Side Snacks' Microbiological Qualities in Ilese-Ijebu Southwestern Nigeria: Implications on Packaging and Bioaerosol Monitoring. *ABC Research Alert* **5**(3): 9 – 18.
- Balaban, N., and Rasooly. A. (2000). Review: staphylococcal enterotoxins. *International Journal of Food Microbiology* **61**:1–10.
- Benson, H.J. (2002). *Microbiological Applications: Laboratory Manual in General Microbiology*. Complete version, 5th edition. McGraw-Hill, New York, pp: 185-1188.
- Biswajit, S., Anil, K..S., Abhrajyoti, G. and Manjusri, B.(2008). Identification and characterization of a vancomycin resistant *Staphylococcus aureus* isolated from Kolkata (South Asia). *Journal of Medical Microbiology* **57**: 72–79.
- Cencil, G.B.T., Karma, M., Rositto, P.V., Morgante, R.A. and Cullor, J.S. (2003). Enterotoxin production by *Staphylococcus aureus* isolated from mastitic cows. *Journal of food protection* **66** (9): 1693-1696.
- Centre for Food Safety (2007). *Microbiological Guidelines for Ready-to-eat Food*, The Centre for Food Safety, Food and Environmental Hygiene Department, Hong Kong. 15 pp
- Cheesebrough, M. (2004). District Laboratory Practice in Tropical Countries. Second Edition part 2. Cambridge press, England, pp: 250-260.
- Chen, T.R., Chiou, C.S. and Tsen. H.Y. (2004). Use of novel PCR primers specific to the genes of *staphylococcal* enterotoxin G, H, I for the survey of *Staphylococcus aureus* strains isolated from food-poisoning cases and food samples in Taiwan. *International Journal of Food Microbiology* **92**: 189-197.

- Christiane, G., Manfred, G. B. and Christiane. W. (2001). Quantification of bacterial transcripts during infection using competitive reverse transcription-PCR (RT-PCR) and Light Cycler RT-PCR. *Clinical Diagnostic and Laboratory Immunology*. **8**: 279-282.
- Esen, R.K and Owusu, E. (2013). Quality of Cooked Foods in Urban Schools in Ghana: Review of Food Borne Diseases and Health Implications, *International Journal of Scientific and Technology Research*. **2**(10):267-275.
- Falola, A.O., Olatidoye, O.P., Balogun, I.O and Opeifa, A.O. (2011). Microbiological Quality Analysis of Meat Pies Sold by Street Hawkers: A Case Study of Mainland Local Government Area of Lagos, Nigeria. *Journal of Medical and Applied Biosciences* **2**: 1- 8.
- Hait, J.M., Tallent, S.M. and Bennett, R.W. (2014). Screening, Detection, and Serotyping Methods for Toxin Genes and Enterotoxins in *Staphylococcus* Strains. *Journal of AOAC International* **97**(4):1078-1083.
- Johnson, W.M., Tyler, S.D., Ewan, E.P., Ashton, F.E., Pollard, D.R. and Rozee, K.R. (1991). Detection of genes for enterotoxins, exfoliative toxins and toxic shock syndrome toxin-1 in *Staphylococcus aureus* by the polymerase chain reaction. *Journal of Clinical Microbiology*, **29**, 426-430.
- Kawo, H.A. and Abdulmumin, F.N. (2009). Microbiological quality of pre-packaged sweets sold in Metropolitan Kano, Nigeria. *Bayero Journal of Pure and Applied Science*. **2**(1): 154-159.
- Kerouanton, A. (2007). Characterization of *S. aureus* strains associated with food poisoning outbreaks in France. *International Journal of Food Microbiology*. **115**: 369-375.
- Kharel, N, Palni, U., and Tamang, I.P. (2016). Microbiological assessment of ethnic street foods of the Himalayas *Journal of Ethnic Foods* **3**: 235 – 241
- Kotzekidou, P. (2013). Microbiological examination of Ready-to-eat foods and Ready-to-bake frozen pastries from University canteens. *Food Microbiology*. **34**: 337-343.
- Madueke, S. N., Awe, S. and Jonah, A. I. (2014). Microbiological analysis of street foods along Lokoja- Abuja expresses way, Lokoja. *American Journal of Research Communication* **2**(1):196-211.
- Mathenge, J.M., Okemo, P.O., Ng'ang'a, P.M., Mbaria, J.M. and Gicheru, M.M. (2015). Identification of enterotoxigenic *Staphylococcus aureus* strains from meat and dairy products by multiplex PCR and reverse passive latex agglutination test in Nairobi, Kenya. *East and Central Africa Medical Journal* **2**: 97-103
- Mbah, M., Ogban, G. I., Konlack, G.D., Useh, M. F., Asuquo, A. E. (2012). The Bacteriological Status of Five Selected Street Vended Cooked Foods in Calabar, Nigeria. *Journal of Pharmacy and Biological Sciences* **2** (4): 25-29.
- Mensah, P., Nicholas, S.L. and Mithani, C.L. (2002). Street foods in Accra, Ghana: How safe are they? *Bulletin of the World Health Organization*. **80** (7): 546-554.
- Nester, E.U., Anderson, D.G., Roberts, E. Jr and Nester MT (2007) *Microbiology: A human perspective* 5th ed. McGraw Hill Companies Inc, Boston, New York 811pp.
- Ochei, K.C., Obeagu, E.I., Aniukwu, V.E. and Mbajiuka, C.S. (2014). The Bacteriology of Fried Ready-To-Eat Foods Sold In Enugu Metropolis, Nigeria. *Journal of Environmental Science, Toxicology and Food Technology*; **8**(1): 81-92.
- Ogugbue, C.J, Mbakwem-Aniebo, C. and Akubuenyi, F. (2011). Assessment of microbial air contamination of post processed garri on sale in markets. *African Journal of Food Science* **5**(8): 503 – 512.
- Oh, S.K., Lee, N. Cho, Y.C., Shin, D., Choi, S.Y. and Koo, M. (2007). Occurrence of toxigenic *Staphylococcus aureus* in Ready-to-Eat Food in Korea. *Journal of Food Protection*, **70**(5):1153–1158.

- Okonko, I.O, Donbraye, E. and Babatunde, S.O.I. (2009). Microbiological Quality of Seafood processors and water used in two different sea processing plants in Nigeria *EJEAFche* **8**(8): 621-629.
- Oranusi, S. and Braide, W. A. (2012). Study of Microbial Safety of Ready-To-Eat Foods Vended On Highways: Onitsha-Owerri, South East Nigeria. *International Research Journal of Microbiology*. **3** (2) 66-71.
- Oranusi, S.U., Oguoma O. I., Agusi, E. (2013). Microbiological quality assessment of foods sold in student's cafeterias *Global Research Journal of Microbiology* **3**(1): 1 –7.
- Ossai, O. S. (2012). Bacteriological Quality and Safety of Street Vended Foods in Delta State. *Nigeria Journal of Biology, Agriculture and Healthcare* **2** (5): 114- 118.
- Owuna, G., Abimiku, R.H., Nkene, I.H., Joseph, G.W. and Ijalana, O.O. (2015). Isolation and Antibiotic Susceptibility of *Staphylococcus aureus* from fresh poultry meat in Keffi metropolis, Nigeria. *International Journal of Research Studies in Biosciences*; **3**(11): 1-5.
- Rath, C.C. and Patra, S. (2012). Bacteriological Quality Assessment of Selected Street Foods and Antibacterial Action of Essential Oils against Food Borne Pathogens. *International Journal of Food Safety* **14**:5-10.
- Rawat, S. (2015). Food spoilage: Microorganism and their prevention. *Asian Journal of Plant science and Research*. **5**(4)47-56.
- Saadati, M., Barati, B., Doroudian, M., Shirzad, H., Hashemi, M., Hosseini, S.M., Chaleshtari, A.R.S., Bahmani, M., Hosseinzadeh, S. and Imani, S. (2011). Detection of Sea, Seb, Sec, Seq genes in staphylococcus aureus isolated from nasal carriers in Tehran province, Iran; by multiplex PCR. *Journal of Paramedical Sciences* **2**(2): 34 - 40.
- Sharma, N.K., Rees, C.E.D. and Dodd, C.E.R. (2000). Development of a single-reaction multiplex PCR toxin typing assay for *Staphylococcus aureus* strains. *Applied Environmental Microbiology*. **6**:1347–1353.
- Su, Y.C., and Wong. A.C.L. (1997). Current perspective on detection of Staphylococcal enterotoxins. *Journal of Food Protection* **60**:195–202.
- Suneetha, C., Manjula, K. and Baby, D. (2011). Quality Assessment of Street Foods in Tirumala. *An Asian Journal of Biological Sciences*. **2** (2): 207 -211.
- Talaro, k. and Talaro, A. (1996). *Foundations in Microbiology* 2nd Edition Mc-Graw Hill Publishers USA. pp. 840-841
- Wallin-Carlquist, N., Marta, D., Borch, E. and Radstrom, P. (2010). Prolonged expression and production of *Staphylococcus aureus* enterotoxin A in processed pork meat. *International Journal of Food Microbiology* **141**:69-74.
- Wogu, M. D., Omoruyi, M. I., Odeh, H. O. and Guobadia, J. N. (2011). Microbial load in ready-to-eat rice sold in Benin City. *Journal of Microbiology and Antimicrobials* **3**(2):29-33.
- Wu, S., Duan, N., Gu, H., Hao, L., Ye, H., Gong, W. and Zhouping Wang, Z. (2016). A review of the methods for detection of *Staphylococcus aureus* Enterotoxins *Toxins* **8**(176); doi:10.3390/toxins8070176
- Xu, Z., Li, L., Chu, J., Peters, B.M. Harris, M.L., Li, B., Shi, L. and Shirliff, M.E. (2011). Development and application of loop-mediated isothermal amplification assays on rapid detection of various types of staphylococci strains. *Food Research International* **47**(2): 166-173.
- Yan, X., Wang, B., Tao, X., Hu, Q., Cui, Z., Zhang, J., Lin, Y., You, Y., Shi, X. and Grundmann, H. (2012). Characterization of *Staphylococcus aureus* strains associated with food poisoning in Shenzhen, China. *Applied Environmental Microbiology*. **78**:6637-6642.
- Zschock, M., Botzler, D., Blocher, S., Sommerhauser, J. and Hamann, H.P. (2000). Detection of genes for enterotoxins (*ent*) and toxic shock syndrome toxin-1 (*tst*) in mammary isolates of *Staphylococcus aureus* by polymerase chain reaction. *International Dairy Journal*, **10**, 569-574.