A study of microbial safety of ready-to-eat foods vended on highways: Onitsha-Owerri, south east Nigeria

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A microbiological safety evaluation was carried out for ready-to-eat foods sold along Onitsha-Owerri highway, South-East Nigeria. Samples were collected from six different check points with intensive business activities - Oba, Okija, Ihiala, Mgbidi, Awomama and Ogbaku. Four hundred and ninety two samples comprising of fourteen different foods were analyzed for total aerobic plate count, coliform, fungi and for common food borne pathogens. The total aerobic plate count was 1.7×10^3- 7.1×10^9 cfu/g. Coliform count range from 2.3×10^1-3.8×10^7 cfu/g. Fungal count range from 1.0×10^1- 1.8×10^6 cfu/g. Approximately 3.9% of the samples analyzed were highly contaminated with total aerobic bacterial count of $> 10^6$ cfu/g. There were no difference in the levels of contamination of samples from the six sampling point. Coliforms, Salmonella spp, *S. aureus, E. coli, B. cereus*, Shigella spp, Enterococci, *A. niger* and Pseudomonas were isolated. Contamination above 10^6 cfu/g food and the presence of potential food borne pathogens could be risky. Consumer's awareness on the dangers of consuming contaminated foods, education of food vendors on food hygiene and application of hazard analysis critical control point (HACCP) is imperative.

Keywords: Coliforms, pathogens, contaminated foods, ready-to-eat foods, HACCP.

INTRODUCTION

Food either raw or cooked, hot or chilled that are ready for immediate consumption at the point of sale without further treatment are generally described as "ready-to-eat (Tsang, 2002). The FAO defined street food as ready-to-eat foods and beverages prepared and/or sold by vendors and hawkers especially in streets and other similar public places (FAO, 1989).

'Migratory' ready-to-eat food vending along highways linking various geographical regions/states of Nigeria is an emerging new form of food vending resulting from high level of unemployment and failed family and community values. The trade is encouraged by travelers who are often delayed or trapped on highways for long hours/days due to failed road infrastructure (Multiple pot holes) coupled with multiple security check points necessitated by increase highway robbery, smuggling

activities through the highways and revenue generation by various government agencies.

A general observation of our society shows a social pattern characterized by increased mobility due to urbanization, large number of itinerant workers and less family or home centered activities resulting in large percentage of the population depending on ready-to-eat foods for employment and food. This situation however, has resulted that food sanitary measures and proper food handling have been transferred from individual, families to the food vendors who rarely enforce such practices (Musa and Akande, 2002; Draper, 1996).

Food vendors do not form a homogenous group, but differ according to various socio-economic and demographic criteria, they have been classified into stationary and ambulatory (Draper, 1996). EPOC (1985) observed that stationary vendors sale their wares from small stalls, kiosks, pushcarts. They operate from selected strategic locations, including bus and trains, markets and shopping areas, commercial districts, outside schools and hospital, residential suburbs,

factories, and construction sites. In some places, the vendors have regular clientele (Powel *et al.,* 1990; Nasinyama, 1992).

The emergence of migratory ready-to-eat food vendors call for concern because, while the stationary and ambulatory vendors can exercise some food safety caution in order to produce safe product for their clients, and for fear that a bad product can lead to low patronage by consumers or outright confrontation by customers because the vendor is known by their fixed station/location/routes. The migratory vendors have no fixed station/location/route. They move to different routes and locations on highways via commercial motorcycles/vehicles, and their movement is dependent on newly identified failed portions on the highways, repair/construction works on the highways, security posts (check points) accident spots on the highways and possibly they move to different locations/routes to deliberately sale bad products.

Ready-to-eat foods have been reported to be easily available, affordable, provide diverse/variable food source, employment and with a potential for improving food security and nutritional status and general social security (Draper, 1996) it is however, a veritable source of food borne pathogen (Abdussalam and Kaferstein, 1993; Arambulo *et al.*, 1994; Mensah, 2002). This study aim to investigate the microbial safety of ready-to-eat foods sold along Onitsha – Owerri highway with a view to proffering solution for effective management of migratory food vendors.

MATERIALS AND METHODS

Source of Samples

Six security check points along Onitsha – Owerri highway were the source of samples collection. These points were chosen because they were observed to have large concentration of vendors with intensive food vending activities throughout the week, from Monday through Sunday and from early hours of the day to late evening. These points include; Oba, Okija, Ihiala, Mgbidi, Awomama and Ogbaku.

Sample Collection

Four hundred and ninety two samples, comprising of fourteen different ready to eat foods were sampled. Six (6) of each food type was obtained from the sampling points. Two samples of each food type was obtained from the sampling points on three (3) different occasions; August, September and October, 2011. Samples were randomly selected without specific order but however,

with caution to pick from different vendors during each sampling. The samples were collected in sterile polyethylene bags adequately labeled and transported to the laboratory for analysis within two-three hours of collection.

Sample Analysis

For analysis 10g of food was blended in sterile blender (Nakai HR-2818, Japan) and homogenized in 90ml sterile distilled water. For Meat pie, Beef sausage roll and Egg roll, equal portion of the contents were blended while each sample of pealed Orange, Walnut (*Tetracarpidium conophprum*), Apple (*Mallus domestica*), was rinsed out in 10ml sterile distilled water. Further ten fold serial dilutions of the resultant homogenates were made to obtain 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} respectively. From these dilutions, aliquots of 0.2ml was inoculated in replicate plates of different media using the spread plate technique. Plate count agar and Nutrient agar (Oxoid) was inoculated for total aerobic plate count and for isolation of diverse microorganisms. The method of Speck (1976) was used for total coliform count. Eosine Methlene Blue (EMB) agar and MacConkey agar (Oxoid) were inoculated for isolation of enterobacteriaceae, Sabouraud-Dextrose agar (Fluka) for fungi, Baird Parker agar (Oxoid) for *Staphylococcus aureus*.

For the isolation of Salmonellae, Selenite-F broth was inoculated for 24 hours pre-enrichment before sub-culturing onto Salmonella-Shigella agar (Fluka). Aliquots of 0.2ml homogenates was inoculated into MacConkey broth for 5 hours incubation before sub-culturing onto Cysteine Lactose Electrolyte Deficient (CLED) agar for typical colonies.

The plates after inoculation were inverted and incubated at 37^0C for 24 hours under aerobic condition for colony formation. Sabouraud Dextrose agar plates were allowed at room temperature 28 ± 2^0C for 24 – 72 hours. After incubation, dilutions with 30 – 300 colonies were selected and counted using colony counter (Stuart Scientific UK).

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, IMVIC test, Motility, Oxidase, Urease and Catalase tests, reaction on TSI and Cultural characteristic on CLED, EMB, MacConkey, Baird Parker and Salmonella-Shigella agar (Hi-media manual, 2003). Confirmatory identification of the bacterial isolates was based on standard biochemical methods (Jolt *et al.,* 1994). Fungal isolates were identified on the bases of their Macroscopic and Microscopic characteristic (Fawole and Oso, 1986; Tsuneo, 2010).

Table 1. Mean total aerobic bacterial count of ready-to-eat foods sold along Onitsh-Owerri, high way

Sample	Sampling Location					
	Oba	Okija	Ihiala	Mgbidi	Awomama	Ogbaku
Okpa	2.1×10^7	1.3×10^9	4.5×10^9	3.4×10^6	5.2×10^6	6.4×10^6
Apple	4.3×10^6	2.5×10^8	3.1×10^7	1.2×10^6	2.2×10^5	4.4×10^6
Wall nut	7.1×10^9	6.3×10^8	1.8×10^8	3.7×10^9	3.5×10^8	8.5×10^7
Meat pie	2.9×10^5	5.6×10^6	8.1×10^5	2.3×10^6	2.5×10^4	4.5×10^6
Pealed Orange	2.4×10^5	5.3×10^5	1.8×10^6	2.4×10^5	6.5×10^4	2.2×10^4
Plantain chips	8.2×10^6	3.4×10^6	2.2×10^5	1.5×10^5	2.7×10^7	5.5×10^3
Aki-na-Ukwa	3.3×10^4	4.2×10^6	5.6×10^6	4.6×10^5	4.3×10^6	3.1×10^5
Egg roll	6.5×10^6	7.1×10^3	6.3×10^5	5.1×10^5	1.1×10^4	7.3×10^3
Doughnut	1.7×10^3	3.1×10^4	5.4×10^5	4.3×10^5	2.5×10^6	3.3×10^4
Elulu-ngwo	2.6×10^7	2.4×10^7	1.3×10^6	4.2×10^7	NA	NA
Sliced Pineapple	1.0×10^7	4.6×10^8	6.6×10^9	9.3×10^6	6.0×10^8	3.6×10^7
Cashew nut	5.9×10^3	8.7×10^5	7.7×10^4	6.8×10^5	7.4×10^6	1.7×10^4
Ground nut	9.8×10^4	1.2×10^4	2.7×10^5	1.1×10^4	6.7×10^4	5.8×10^5
Beef sausage roll	3.4×10^4	6.8×10^4	4.6×10^4	3.8×10^5	4.8×10^5	6.6×10^4

NA= Not analyzed

Table 2. Mean coliform count of ready-to-eat foods sold along Onitsha-Owerri, highway.

Sample	Sampling Location					
	Oba	Okija	Ihiala	Mgbidi	Awomama	Ogbaku
Okpa	2.1×10^5	1.8×10^6	5.5×10^6	6.3×10^5	3.2×10^4	2.7×10^4
Apple	3.1×10^5	3.0×10^6	3.3×10^6	1.2×10^5	1.1×10^2	5.4×10^2
Wall nut	6.4×10^6	7.1×10^5	4.9×10^6	3.8×10^7	4.1×10^5	7.2×10^6
Meat pie	1.4×10^2	2.3×10^2	3.1×10^3	3.3×10^2	1.3×10^2	2.5×10^4
Pealed Orange	2.3×10^1	3.3×10^2	1.7×10^3	2.8×10^1	2.1×10^2	2.4×10^2
Plantain chips	4.1×10^3	1.2×10^3	1.1×10^3	3.0×10^3	1.3×10^3	2.7×10^3
Aki-na-Ukwa	2.3×10^2	2.2×10^3	7.1×10^4	1.5×10^2	3.3×10^3	1.1×10^3
Egg roll	6.5×10^6	7.1×10^3	6.3×10^5	5.1×10^5	1.1×10^4	7.3×10^3
Doughnut	3.2×10^1	1.1×10^4	4.4×10^5	8.3×10^4	2.7×10^4	2.1×10^4
Elulu-ngwo	2.5×10^4	4.2×10^3	2.5×10^5	3.1×10^4	NA	NA
Sliced Pineapple	2.0×10^6	8.2×10^6	2.2×10^7	8.3×10^5	3.3×10^7	3.4×10^6
Cashew nut	1.8×10^3	6.4×10^2	4.8×10^2	3.4×10^3	3.7×10^3	5.3×10^4
Ground nut	4.3×10^4	1.1×10^4	3.3×10^2	3.5×10^2	4.4×10^3	4.6×10^3
Beef sausage roll	3.3×10^2	3.2×10^2	4.3×10^3	1.9×10^3	2.4×10^2	3.6×10^3

NA= Not analyzed

RESULTS

Microbiological quality of food samples on the bases of total aerobic plate count is shown in Table 1. All the samples are contaminated, however, sliced Pineapple (*Ananas comosus*), Okpa (pudding/bean cake product of *Vigna subterranean* (L.) Verdc./ (Bambara groundnut) and Apple (*Mallus domestica* Borkh) had higher counts. There is no difference in contamination rate of samples from the six sampling locations. Table 1 also reveal that apart from Okija, only two to four food items obtained from the different locations had counts > 10^6cfu/g.

Table 2 shows the coliform count of the samples to be high, except however, for Ground nut, Cashew nut and beef sausage roll. Okpa, sliced pineapple, egg roll and Wall nut (*Tetracarpidium conophorum*) had the highest coliform counts. Fungal count was higher in Wall nut, Aki-na-Ukwa (roasted African bread fruit "Ukwa" (*Treculia Africana*) mixed with palm kernel "Aki" (*Elaeis guineensis*) or coconut "Akuoyibo" (*Cocos nucifera*), Plantain chips, Elulu-ngwo "Akpa-ngwo" (Larvae(raffia palm) often fried and sauced) and Groundnut and cashew nut (Table 3). The microorganisms isolated are shown in Table 4. Pathogens were isolated in some of the foods while *B.*

Table 3. Mean fungal count of ready-to-eat foods sold along Onitsha- Owerri, highway.

Sample	Sampling Location					
	Oba	Okija	Ihiala	Mgbidi	Awomama	Ogbaku
Okpa	5.0×10^2	3.0×10^3	1.1×10^3	8.0×10^1	1.4×10^1	1.6×10^2
Apple	1.1×10^2	2.5×10^4	4.1×10^4	3.0×10^3	5.2×10^2	1.1×10^2
Wall nut	3.5×10^5	3.1×10^5	9.0×10^4	1.9×10^5	1.8×10^6	4.3×10^4
Meat pie	1.0×10^1	2.1×10^1	4.1×10^2	1.2×10^1	3.2×10^2	2.0×10^1
Pealed Orange	1.1×10^2	4.2×10^3	1.7×10^2	2.1×10^2	4.5×10^1	1.2×10^2
Plantain chips	3.3×10^4	4.0×10^5	2.0×10^4	2.7×10^4	2.8×10^5	4.8×10^5
Aki-na-Ukwa	2.2×10^3	1.0×10^5	6.6×10^5	5.4×10^4	2.0×10^4	2.2×10^5
Egg roll	1.0×10^2	1.1×10^3	2.1×10^2	1.1×10^3	3.2×10^2	1.3×10^1
Doughnut	1.0×10^2	2.1×10^2	1.0×10^2	3.3×10^1	2.3×10^2	1.1×10^2
Elulu-ngwo	1.6×10^4	2.3×10^5	1.4×10^5	1.2×10^5	NA	NA
Sliced Pineapple	3.3×10^4	3.5×10^5	3.0×10^5	5.6×10^5	6.3×10^4	2.6×10^3
Cashew nut	4.2×10^4	6.1×10^4	8.2×10^5	1.0×10^5	3.4×10^4	3.0×10^2
Ground nut	2.5×10^4	1.8×10^4	4.3×10^4	1.0×10^3	3.4×10^4	2.6×10^2
Beef sausage roll	1.2×10^2	2.3×10^1	2.2×10^2	1.6×10^2	2.3×10^2	2.4×10^3

NA= Not analyzed

Table 4. Microorganisms isolated from foods sold along Onitsha- Owerri highway, South-East, Nigeria

Food sample	Organisms isolated
Okpa	*E. coli, S. aureus*, *B. cereus*, *B. subtilis Shigella sonnei,* Bacillus spp, Penicillium spp, *A. niger, P. cepacia*
Egg roll	*E. cloacae, P. aeruginosa*, *S. aureus*, Bacillus spp, Proteus spp, Penicillium spp
Doughnut	*C. freundii*, Rhizopus spp, Klebsiella spp, *B. cereus*, *S. aureus*, *E. coli*, *S. epidermides*, Penicillium spp, *Aspergillus niger*
Meat pie	*S. aureus*, *P. putida*, *K. pneumoniae*, Acinetobacter spp, Bacillus spp, *Aspergillus niger*
Beef sausage roll	*E. coli*, *S. aureus*, *B. cereus*, Bacillus spp, Mucor spp, Aspergillus spp.
Elulu-ngwo	Salmonella spp, *B. Licheniformis, B. subtilis,* Aeromonas spp, Penicillium spp, *S. aureus* Aspergillus,
Plantain chips	*P. mirabilis*, *S. aureus, S. epidermidis, E. agglomerans*, Bacillus spp, Mucor spp*, A. niger,* Klebsiella spp
Apple	*E. carotovora,* Bacillus spp, Aeromonas, Salmonella spp, *S. aureus, A. niger,* Fusarium spp.
Pealed Orange	*S. aureus, A. niger, B. cereus,* Pseudomonas*, E. coli,* Bacillus spp
Sliced Pineapple	Erwinia spp*,* Bacillus spp, *E. coli,* Aeromonas, Salmonella spp, *A. niger,* Fusarium spp, *P. aeruginosa*, *S. aureus,* Saccharomyces spp
Wall nut	*S. marcescens,* Enterococcus*, S. aureus,* Bacillus spp, Aeromonas, *E. coli,* Salmonella spp, *S. aureus, A. niger,* Fusarium spp, *P. aeruginosa,*
Ground nut	Enterobacter spp, *S. liquefaciens, B. Megaterium,* Aspergillus, Fusarium, Proteus spp, *S. aureus, S. epidermidis*
Cashew nut	*K. pneumonia,* Pseudomonas, Penicillium spp*, A. niger, S. aureus,* Bacillus spp
Aki-na-Ukwa	*S. aureus, S. saprophiticus, B. cereus, P. aeruginosa,* Cladosporium, Klebsiella spp, Penicellium spp

cereus, *S. aureus*, pseudomonas and *E. coli* are the predominant microorganisms. Salmonella and Shigella spp was identified in five (5) samples.

DISCUSSION

Despite the high level of contamination of the ready-to-eat foods in this study, most of the sampled foods contain total aerobic plate counts of $\leq 10^6$cfu/g. These foods are therefore considered fit for human consumption (ICMSF, 1974; FAO/WHO, 2005). The high microbial load recorded for Okpa could be associated with the production and handling of the product. Okpa is often wrapped in dried banana leaves that are not cleaned to remove microbial load (Okeke *et al*, 2008). Left over products are normally re-heated for subsequent day sales.

Wall nut (*Tetracarpidium conophorum*) by definition of ready-to-eat food (Gilbert *et al.,* 2000) is not to be considered as a ready-to-eat food by virtue of the fact that the shell need to be removed for the content to be consumed. The shell is normally removed with the teeth and contaminants are easily swallowed. Poor handling of Wall nut and the natural microflora could have contributed to the high level of contamination.

Contaminants may invade the interior surfaces of fruits during pealing, slicing, trimming, packaging and from handling by the vendor. These could have accounted for the high microbial load in sliced Pineapples. Lack of storage facilities could have heightened the chances of contamination (SCF/CS/FMH/SURF/Final, 2002; Baro *et al.*, 2007). The products are maintained throughout the day under the intense heat of the sun, this encourages proliferation of contaminants. Fruits have been associated with outbreaks of food borne diseases in many countries Jay, 1996; De-Rover, 1998; Kaplan and Campbell, 1982; Reis *et al.*, 1990; Chukwu, *et al.*, 2010; CDC, 1979; CDC, 2009). Organisms implicated in ford borne diseases are numerous and diverse bacteria, fungi, viruses and parasites (Jay, 1996; De-Rover, 1998; Chukwu *et al.*, 2010).

The extensive handling of Plantain chips during packaging, and the staking and saucing of Elulu-ngwo could have contributed immensely to the level of contamination. The ICSMF (1974) identified foods with counts > 10^6cfu/g as unacceptable for consumption. Okpa, Elulu-ngwo, sliced Pineapple therefore contain unacceptable levels of microorganisms.

Coliforms are indicator organisms; their presence in ready-to-eat foods portends possible danger. Coliform counts of $\geq 10^4$ recorded for Okpa, sliced Pineapple, some Egg roll, Apple and Elulu-ngwo calls for strict adherence to standard food practices and effective HACCP application.

The fungal counts for Wall nut, Plantain chips, Aki-na-Ukwa, Elulu-ngwo, Cashew nut and Ground nut are of the order 10^5, mishandling of these products could lead to proliferation of fungi beyond acceptable limits.

E. coli, S. aureus, B. cereus, Shigella, Salmonella and Pseudomonas 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.*, 2006; 2007; Tambeker *et al.,* 2008; Yadav *et al.,* 2011).

This study reveals that while some of the foods vended by these migratory food vendors are contaminated beyond acceptable microbiological limits, most of the foods are of acceptable microbiological standard. Foods vended along the highways supply the energy and nutrient need of the travelers and create employment to the teaming unemployed youths (vendors) however, the danger associated with migratory food vendors of unknown identity, untrained in food safety and of unknown educational background is enormous and must be discouraged. The danger these young and feature leaders (age ≥ 10 to ≤ 45) are exposed to on the highway can best be imagined if and when one observe them meandering between vehicles on motion and in most cases run after vehicles for several meters to get their wares sold.

Families and communities must rise to their responsibility of training their wards. Government at all levels must assume their social responsibility of youth empowerment, aggressive road/general infrastructure maintenance. This will reduce/eliminate unemployment, bad roads, insecurity on the highways and multiple road blocks (security check points). Street food vending in Nigeria cannot be stopped in the very near future, Government must therefore create enabling safety regulations. This will go a long way to regulate vendors. Government can develop standard relaxation points along the highways where motorists can stop-over. The zeal with which the vendors go after vehicles on motion can be harnessed effectively to improve micronutrient deficiency (Barth, 1983; Atkinson, 1992; ACC/SCN, 1992; Rossi-Espagnat *et al.*, 1991) if and when micronutrient fortified products are to be sold by these vendors to travelers in stop over relaxation points along the highway.

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