MICROBIAL SAFETY OF READY-TO-EAT FOODS SOLD IN OWERRI MUNICIPAL COUNCIL, IMO STATE, NIGERIA

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Abstract

A microbiological safety evaluation was carried out for ready-to-eat foods within Owerri Municipal Council of Imo state, Nigeria. Thirty-five samples of five different foods were collected from five different locations representing the five communities in Owerri Municipal Council (Amawom, Umuororonjo, Umuodu, Umuonyeche and Umuonyima). The samples were analyzed for total aerobic plate count, Coliform, Salmonella spp, Shigella spp, Vibro cholera and Staphylococcus aereus. The isolates were identified using biochemical characteristics and analyzed using the one-way- ANOVA test. Most of the samples yielded significant ($P<0.05$) bacterial growth (10^6 cfu/g), although food samples from open street had higher growth than those from standard fast food cafeterias. Bean cakes from Umuonyeche had the highest total aerobic count ($7.6x10^6$), while maize pudding from Amawom had the lowest ($2.5x10^6$). There was statistically significant difference ($P<0.05$) in the microbial contamination of bambara groundnut puddings from the five sampling locations, but samples of bean cakes and maize puddings showed no significant difference ($P>0.05$). Coliforms, Salmonella spp, Shigella spp and Staphylococcus aereus were isolated from most of the samples. Interestingly, Vibro cholera was not isolated in most of the samples. Among the fungal isolates were: Aspergillus fumigatus, Acremonium spp, Penicillium spp, Fusarium spp, Aspergillus niger, Cladosporium spp, Aspergillus flavus, Candida spp and zygomyces spp. Contamination above 10^6 cfu/g of food sample and the presence of potential food borne pathogens have grave public health concern implications. Consumers need to be sensitized on the dangers of street sold ready-to-eat foods. Education of food handlers on food and personal hygiene and application of hazard analysis critical control point principles (HACCP) is very necessary.

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

Introduction

The busy and hectic life schedule has opened the way for the fast food industry in most parts of the world. The traditional or conventional way of cooking is over and the fast food joints are visible everywhere. Fast food does not only include the exotic fast food items like pizza, burger or French fries but also traditional fast foods like akara (bean cakes), moimoi (beans pudding), dodo (fried plantain), agidi (maize pudding) etc. In Nigeria, vended food is intimately connected with take-away, junk food, snacks, and fast food; it is distinguished by its local flavour and by being purchased on the street, with or without entering any building. Both take-out and fast food are often sold from counters inside buildings (Chukuezi, 2010). In spite of numerous advantages offered by street foods, there are also several health hazards associated with this sector which arise majorly from traditional processing and packaging, improper handling, temperature, poor personal hygiene of food vendors and so on.

A high number of foods sold in our communities are contaminated to a large extent with pathogenic micro organisms. Foods sold near polluted environments are prone to contamination by pathogenic micro organisms. Documented evidences have continued to link pathogenic micro organisms

in food to incidences of food borne diseases and intoxications which are due mainly to poor personal hygiene and insanitary environmental condition (Aboloma, 2008; Shamsudden &Ameh, 2008; Kawo & Abdulmumin, 2009; Kabiru et al., 2013). Again in developing countries such as Nigeria, there are serious concerns about sanitation of ready-to-eat foods, particularly as potable water is seldom available at preparation venues and fast food stands. It is in view of this that this study was conducted to evaluate the microbial quality of some ready-to-eat foods vis-à-vis enumeration of aerobic mesophilic bacteria and fungi and also determine the incidence of some enteropathogenic bacteria on the foods sold in Owerri Municipal with a view to providing data on the incidence of such pathogenic micro organisms for necessary action on the part of food sellers, consumers and authorities.

Materials and Method

Area of study

The study area was the five indigenous communities of Owerri Municipal Council (Owerre Nchi ise) Vis: Umuororonjo, Amawom, Umuonyeche, Umuodu and Umuoyima.

Sample Collection

A total of 35 food samples were purchased comprising five of each of these food types: meat pie, bambara groundnut pudding, bean cakes, and maize pudding. Samples were taken from both fast food cafeterias and the open street .They were collected in sterile polythene bags and transported to the laboratory within three hours of collection in cool boxes with ice.

Sample Analysis

Twenty- five grammes of each sample was weighed and homogenized by blending in 225 ml of sterile buffered peptone water. Further ten-fold serial dilution of the resultant homogenates were made to obtain 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} respectively. The procedure was repeated for each sample and the blender was carefully cleaned and disinfected in between samples to prevent any cross contamination.

Aliquots (0.1ml) of each (10^{-2}, 10^{-3}) of each sample were used to inoculate Nutrient agar (for Total aerobic plate count), Eosin-Methylene Blue Agar (for Coliform count), Salmonella-Shigella Agar (for Salmonella spp and Shigella spp count),Thiosulphate-Citrate-Bile-Salt Agar (for isolation of Vibro cholera), Cystine Lactose Electrolyte Deficient Agar (for Staphylococcus aureus count) and Sabouraud Dextrose Agar (for isolation of fungi) by the spread plate method. These were then incubated at 37°c for 24 hours for bacteria and 25°c for 78-96 hours for fungi. The counts for each plate were expressed as colony forming unit per gramme of sample homogenate (cfu/g). Morphological attributes of the colonies on the media were observed; characteristic colonies on the different media were isolated and purified by repeated sub-culturing on Nutrient Agar. Pure cultures were stored on agar slants at 4°c for further characterization.

Identification of Isolates

Cultural characteristics such as shape, colour, size and consistency were carried out; Isolates were Gram stained and subjected to appropriate biochemical tests which include: Sugar utilization, Indole test, IMVIC test, Motility, Oxidase, Urease and Catalase tests. Fungal isolates were identified on the bases of their Macroscopic and Microscopic characteristics (Fawole & Oso, 1986; Tsuneo, 2010).

Statistical Analysis

Statistical analysis was performed using SPSS version 19. One-way ANOVA was used to compare the bacterial counts in various food types and bacterial count of ready-to-eat foods from standard fast food cafeterias and the open street. The mean difference was considered significant at $p<0.05$.

Results

Total Aerobic Bacteria Count (cfu/g) of Maize Pudding Samples

All the maize pudding samples from the different locations had total aerobic count of 10^5 cfu/g except the sample from Umuodu which had a significantly higher count of 10^6 cfu/g (Table 1). *E.coli* was isolated from all the locations in the order of 10^4 cfu/g. However, samples from Umuoyeche and Umuoyima had no Coliform growth (Table 1). *Klebsiella spp* was isolated from only maize pudding samples from Umuororonjo. No *Klebsiella spp* growth was observed in sample from other locations (Table 1). No *Vibro cholera* growth was observed in maize pudding samples from the different locations (Table 1). *Staphylococcus aureus* was isolated from maize pudding samples from Amawom and Umuoyeche. The other locations recorded no *Staphylococcus aureus* growth (Table 1).

TABLE 1: Total Aerobic Bacteria Count (cfu/g) of Maize Pudding Samples

Location	Nutrient Agar (Total aerobic bacteria)	Eosin Methylene Blue Agar (E.coli)	Eosin Methylene Blue Agar (Klebsiella)	Salmonella/ Shigella Agar (Salmonella spp)	Thiosulphate Citrate Bile Salt Agar (Vibro cholera)	Cystine Lactose Electrolyte Deficient Agar Staphylococcus aureus
Amawom Maize Pudding (Agidi)	2.5×10^5	2.0×10^4	NG	2.3×10^4	NG	7.0×10^4
Umuororonjo Maize Pudding (Agidi)	4.8×10^5	1.3×10^4	1.8×10^4	NG	NG	NG
Umuodu Maize Pudding (Agidi)	1.06×10^6	1.0×10^4	NG	NG	NG	NG
Umuoyeche Maize Pudding (Agidi)	3.3×10^5	NG	NG	NG	NG	3.0×10^4
Umuoyima Maize Pudding (Agidi)	8.5×10^5	NG	NG	1.0×10^4	NG	NG

Source: Authors Field Work, 2015

Total Aerobic Bacteria Count (cfu/g) of Bean Cake Samples

Bean cake (akara) samples from the different locations were significantly contaminated with aerobic bacterial counts in the order of 10^6 cfu/g (Table 2).This count is not tolerable in ready-to-eat foods (ICMSF,1996). Coliforms were isolated from all the food samples from the different location though in low counts (10^2cfu/g) (Table 2). Also bean cake samples from the differents locations had low Klebsiella spp growth in the order of 10^2 cfu/g (Table 2). Except for bean cake samples from Umuodu which had a Salmonella spp count of 1.0×10^3 ,all the other locations had higher counts in the order 10^3 cfu/g (Table 2). Vibro cholera was isolated from only two locations: Umuororonjo and Umuodu. All the other locations had no Vibro cholera growth (Table 2).

Apart from bean cake samples from Umuoyeche and Umuoyima that had significantly higher counts of Staphylococcus aureus in the order of 10^5, cfu/g, samples of bean cakes from all the other different locations had lower growth of Staphylococcus aureus ($\leq 10^2$cfu/g)(Table2).

Table 2: Total Aerobic Bacteria Count (Cfu/g) OF Bean Cake Samples

Location	Nutrient Agar (Total aerobic bacteria)	Eosin Methylene Blue Agar (E.coli)	Eosin Methylene Blue Agar (Klebsiella)	Salmonella/ Shigella Agar (Salmonella spp)	Thiosulphate Citrate Bile Salt Agar (Vibro cholera)	Cystine Lactose Electrolyte Deficient Staphylococcus aureus
Amawom Bean Cakes (Akara)	2.08×10^{6}	1.0×10^{4}	9.0×10^{3}	1.2×10^{4}	NG	2.5×10^{3}
Umuoronjo Bean Cakes (Akara)	1.2×10^{6}	9.0×10^{3}	1.2×10^{3}	5.0×10^{4}	1.0×10^{3}	4.0×10^{3}
Umuodu Bean Cakes (Akara)	3.05×10^{6}	7.0×10^{3}	1.0×10^{3}	1.0×10^{3}	1.0×10^{3}	1.0×10^{3}
Umuoyeche Bean Cakes (Akara)	7.6×10^{6}	3.0×10^{3}	4.3×10^{3}	6.8×10^{4}	NG	1.3×10^{4}
Umuoyima Bean Cakes (Akara)	2.31×10^{6}	1.0×10^{3}	6.0×10^{3}	3.0×10^{4}	NG	2.6×10^{3}

Source: Authors Field Work, 2015

Total Aerobic Bacteria Count (cfu/g) of Bambara Groundnut Pudding Samples

Bambara groundnut pudding samples from the different locations were significantly contaminated with aerobic bacterial counts in the order of 10^{6} cfu/g (Table 3). Low counts of Coliforms were observed in all the food samples from the different locations ($<10^{3}$ cfu/g) (Table 3). Similarly, Klebsiella spp was isolated from bambara groundnut pudding samples from the different locations in low counts ($<10^{3}$ cfu/g) (Table 3). Except Umuodu, all the other locations had higher counts of Salmonella spp in the order of 10^{4} cfu/g (Table 3). Again, Vibro cholera was isolated from only two locations: Umuororonjo and Umuoyima. All the other locations had Vibro cholera growth in the order of 10^{3} cfu/g (Table 3).

Except for bambara groundnut pudding samples from Umuoyeche which had a Staphylococcus aureus counts in the order of 10^{4} cfu/g, samples from the other locations had lower Staphylococcus aureus count ($<10^{3}$ cfu/g)(Table3).

Table 3: Total Aerobic Bacteria Count (cfu/g) of Bambara Groundnut Pudding Samples

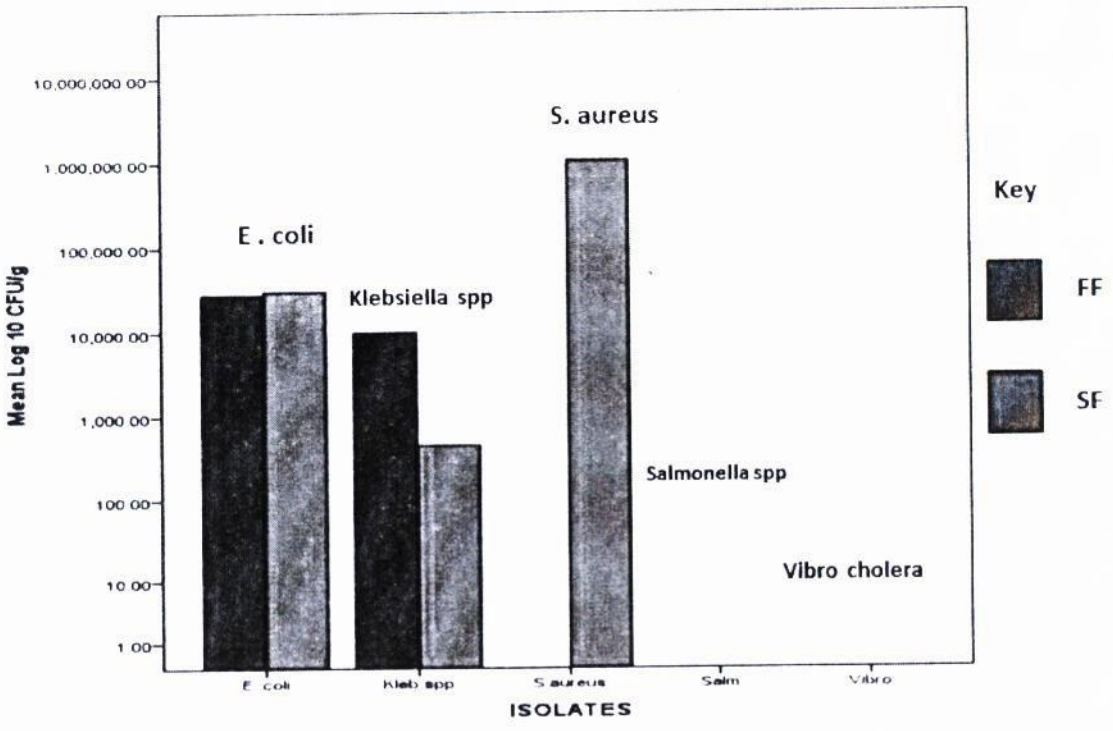
Location	Nutrient Agar (Total aerobic bacteria)	Eosin Methylene Blue Agar (E coli)	Eosin Methylene Blue Agar (Klebsiella)	Salmonella / Shigella Agar (Salmonella spp)	Thiosulphate Citrate Bile Salt Agar (Vibro cholera)	Cystine Lactose Electrolyte Deficient Staphylococcus aureus
Amawom Bambara Groundnut Pudding (Okpa)	6.2×10^{6}	3.7×10^{2}	5.5×10^{2}	6.4×10^{4}	NG	5.3×10^{2}
Umuorogo Bambara Groundnut Pudding (Okpa)	1.72×10^{6}	3.0×10^{2}	7.0×10^{2}	5.7×10^{4}	1.0×10^{3}	1.25×10^{2}
Umuodu Bambara Groundnut Pudding (Okpa)	1.75×10^{6}	2.0×10^{2}	1.0×10^{2}	NG	NG	1.9×10^{2}
Umuoyeche Bambara Groundnut Pudding (Okpa)	1.47×10^{6}	5.0×10^{2}	4.0×10^{2}	2.9×10^{4}	NG	4.1×10^{2}
Umuoyima Bambara Groundnut Pudding (Okpa)	7.9×10^{5}	2.0×10^{2}	2.0×10^{2}	2.1×10^{4}	1.0×10^{3}	2.1×10^{4}

Source: Authors Field Work, 2015

Total Aerobic Bacteria Count (cfu/g) of Beans Pudding (moi-moi) Samples

A similar comparison was made for beans pudding (moi-moi) samples from standard fast food cafeterias and those from the open street. There was no statistically significant difference ($P>0.05$) in the total aerobic bacterial loads of beans pudding (moimoi) from standard fast food cafeterias and the open street. However, E.coli, Staphylococcus aureus and Salmonella spp counts in beans pudding from the open street were higher than counts from standard fast food cafeterias (Figure 1).Klebsiella spp count in samples from standard fast food cafeterias was higher than that from the open street (Figure 1).There was no Vibro cholera detected in beans pudding samples from both sampling points (Figure 1).

Figure1. Bacterial Contamination of Beans Pudding from Standard Fast Food Cafeterias and the Open Street



Legend: FF, fast food; SF, Open street food

Figure 2. Bacterial Contamination of Meat Pie From Standard Fast Food Cafeterias and the Open Street

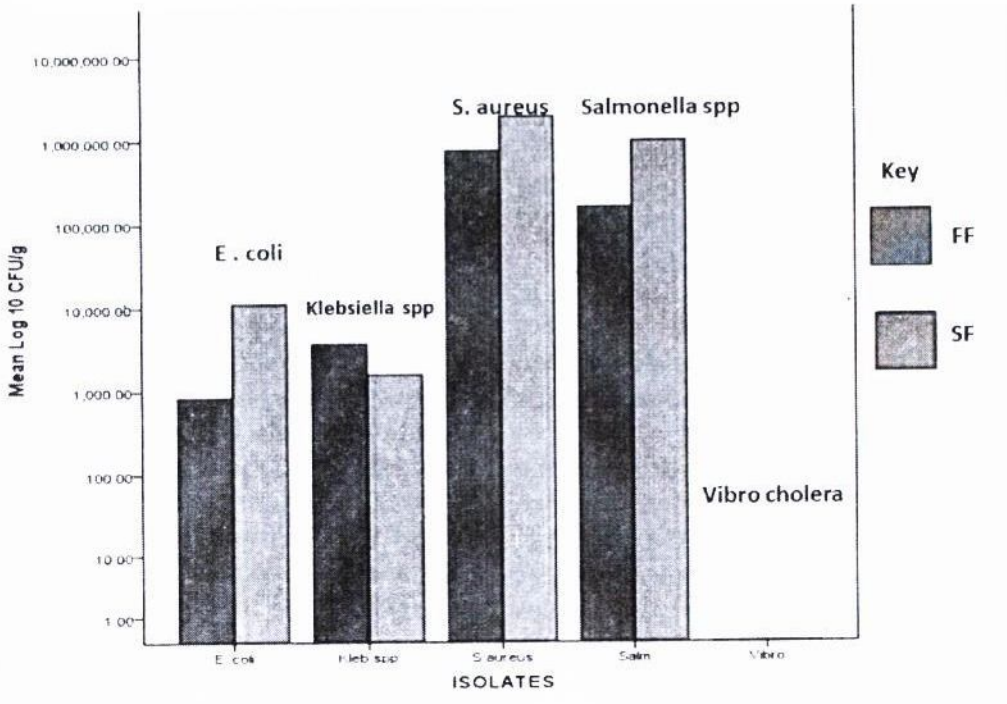


Table 6: Fungal isolates in the various food samples examined

Food sample	Organisms isolated
Bean cake (Akara)	Aspergillus fumigatus, Acremonium spp, Penicillium spp, Fusarium spp, Aspergillus niger.
Bambara groundnut pudding (Okpa)	Aspergillus fumigatus, Cladosporium spp, Fusa Acremonium spp, Penicillium spp , Fusarium spp , Aspergillus flavus, Candida spp
Street meat pie	Aspergillus fumigatus, Fusarium spp
Fast food meat pie	Penicillium spp, Aspergillus flavus, Candida spp, Zygomyces spp
Street beans pudding (moimoi)	Aspergillus fumigatus, Fusarium spp
Fast food beans pudding (moimoi)	Aspergillus fumigatus, Fusarium spp, and Candida spp
Maize pudding (Agidi)	Aspergillus fumigatus, Fusarium spp , Acremonium spp , Aspergillus flavus, Candida spp, Zygomyces spp, Aspergillus niger

Discussion

In this study, most of the sampled foods showed total aerobic plate counts of $> 10^5$ cfu/g. Hence, the food samples can be considered to be of unacceptable microbial quality (ICMSF, 1996). The high microbial load recorded for street foods could be associated with lack of sources of running water, refrigeration facilities, unsanitary environment and post production operation and personal hygiene of the food handlers. Bukar Uba & Oyeyi (2010) found that ready-to-eat rice sold on the street of Kano contained more micro organisms compared to that sold in standard fast food cafeterias.

Bambara groundnut pudding (okpa) and moimoi (from fast food) had relatively higher total aerobic plate count. This could have been as a result of massive contamination from the leaves in which they are wrapped.

Okpa is often wrapped in dried banana leaves that are not pre-treated to remove microbial load (Okeke,Eneobong, Uzuegbunam, Ozioko & Kuhnlein, 2008).

Maize pudding (agidi), (except for samples from Umuodu), had lower microbial load (10^5 cfu/g) than the other food samples. Microbial load of $\leq 10^5$ /g is tolerable (ICMSF. 1996). This could be associated with the long temperature and time involved in its production. E.coli, Staphylococcus aureus, Salmonella spp and Klebsiella spp were isolated from many of the food samples indicating poor hygiene and sanitation. The isolation of these organisms corroborated the findings of Oranusi, Oguoma & Agusi, (2013) in which these organisms were implicated in ready-to-eat foods.

The prevalence of Staphylococcus aureus in the food samples is due to human contact and this suggests poor personal hygiene practices of the vendors because the organism is a normal flora of the skin and nasal passage (Garret, 1988; Nichols Little, Mithani & De Louvois, 1999). The toxins produced by Staphylococcus aureus is heat stable and may not be destroyed even by heating. Foods that are handled frequently during preparation are prime targets for Staphylococcus aureus contamination (Ghosh, Wahi,Kumar & Ganguli, 2007). Salmonella spp and E.coli are of fecal origin and have been implicated in many food borne diseases (Oranusi Galadima, Umoh & Nwanze, 2007). Their presence in the food samples is an indication of possible fecal contamination of food, water or food workers and poor hygiene practices (Tambeker, Shasat, Duradkar, Rajanka & Banginwar, 2007). Coliform counts of 10^4 cfu /g recorded for some of the maize pudding and meat pie samples calls for adherence to

standard food practices and effective HACCP application. The presence of fungal isolates in the order of 10^4 cfu/g in most of the food samples could inadvertently lead to proliferation of these organisms above acceptable limits. The occurrence of these organisms could be due to the fact that they are spore formers and their heat resistant spores made it through the critical control points in processing of the food samples. Fungal contamination of food may result from inadequate heating or secondary contamination through contact with contaminated equipment and utensils (Oranusi, et al., 2013). The findings in this research revealed that some ready-to-eat foods (meat pie, bean cakes (akara), maize pudding (agidi), beans pudding (moimoi) and bambara groundnut pudding (okpa), sold within Owerri Municipal Council were contaminated beyond acceptable microbial limits. This contamination is an indication of inadequate processing and poor handling practices which have grave public health implications. The application of Good Manufacturing Practices (GMP) and Hazard Analysis critical Control Point (HACCP) in food processing and production as well as health education could help improve food safety.

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