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Bacteriological Quality of Foods and Water Sold by Vendours and in Restaurants in Nsukka, Nigeria: Assessment of Coliform Contamination

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Abstract: About 66 food samples representing 10 food types (beans, yam, abacha, okpa, moimoi pear, cassava-foofoo, rice, agidi and garri) and 10 water samples obtained from food vendors and restaurants in Nsukka were examined for bacteriological quality (coliform contamination) using the Most Probable Number (MPN) and Lactose Fermentation Count (LFC)/*Escherichia Coli* Count (ECC) methods. The food samples examined exhibited varying degree of contamination. The percentage contamination ranged from 50-96.3, 33.3-100 and 16.7-100% for the MPN, LFC and ECC, respectively. Of this, pear had the highest percentage contamination (96.3, 100 and 100) for the MPN, LFC and ECC methods used, respectively. The bacterial count was expressed as Geometric Mean Count (GMC). The GMC (Log_{10}CFU mL^{-1} or g) for the LFC and ECC ranged from 5.32-9.26 and 4.46-7.81, respectively. Abacha had the highest coliform count in both the LFC (9.26) and ECC (7.81). The MPN count ranged from 1-180. Expectedly, the Analysis of Variance (ANOVA) showed significant difference ($p<0.05$) in bacterial count amongst the various food samples, however there was no significant difference ($p>0.05$) in the three methods used for the coliform enumeration. *Escherichia coli* and *Klebsiella pneumonia* were the two major coliforms identified of the 98 coliform isolates obtained from the various food samples of which 78 (79.6%) were of human origin having multiplied at 44°C. The level of coliform contamination in the food samples from vendours and restaurants were above limits. Thus, this study calls for stringent supervision/implementation of food safety practices and regular education on food and personal hygiene.

Key words: Coliform, contamination, hygiene, geometric mean count, rural, enumetration

INTRODUCTION

Food and most especially water have been described as vehicles for the dissemination of microbial diseases among which are those caused by coliforms (Ifediora *et al*., 2006). Coliforms are gram negative facultative anaerobes that ferment lactose to produce acid and gas. These include *Escherichia coli*, *Klebsiella pneumonia* and *Enterobacter*. Consumption of food or water contaminated with these coliforms could result in diseases such as typhoid fever, diarrhea and gastroenteritis.

Sources of these contaminations include poor environmental sanitation, poor personal hygiene of food handlers and poor preparation and storage. Studies have shown that foods are sometimes exposed at improper temperatures, improperly handled by food vendours and sold at dirty and unhygienic environment (WHO, 2001, 2003; Agbodaze *et al*., 2005; Muinde and Kuria, 2005; Ghosh *et al*., 2007). Most of the vendours had no formal education and therefore lack knowledge on proper food handling and their role in transmission of pathogens. Nsukka is a rural town in Enugu State in the South-Eastern Nigeria and generally lack access to good pipe borne water, drainage systems and good waste disposal facilities.

These lack of social amenities have contributed to major public health problems. Keeping this in view, report the bacteriological quality of food sold by vendours and in restaurant in Nsukka town by enumerating the incidence of coliform in the foods and thus ascertaining the level of hygiene involved in such food preparations.

MATERIALS AND METHODS

Sample collection: The study was carried out in Nsukka Township of Enugu State, Nigeria both within and outside

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the university campus. A total of 66 samples (representing ten food types) and ten water samples (from various sources) obtained from food vendors and restaurants were collected. All food samples were collected in sterile disposable containers (Sterilin). Each sample was properly identified with a number code, subject name, type of food and condition of food (fresh or reheated). Samples were sent to the laboratory in a cold box containing ice-blocks within 2 h. The different types of food collected are shown in Table 1.

Bacteriological analysis: Three methods were used in enumeration of bacterial level; Lactose Fermentation Count (LFC), the *E. coli* Count (ECC) and the Most Probable Number (MPN) Approximately 2 g of food each food sample was homogenized in 20 mL of sterile saline and the homogenate diluted in 10 fold series. For the LFC, a 0.1 mL was taken from the 10^{-5} dilution and innoculated in duplicate plates on MacConkey Agar (MCA) using a sterile glass rod spreader. Incubation was carried out at 37°C for 48 h on one plate and the second plate at 44°C for 24 h. The *E. coli* Count (ECC) enumeration was the same as the LFC above except that 10^{-4} dilution was used to inoculate Eosin Methylene Blue Agar (EMBA) plates instead of MacConkey agar. A 20 g of food samples was washed in 200 mL of sterile saline and was used to inoculate the different strengths of MacConkey broth for the MPN count (Cheesbrough, 1993). Colonies were counted and identity was confirmed by standard bacteriological methods (Collins and Lyne, 1980).

Statistical analysis: Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS), Fishers Least Significant Difference (F-LSD) and student's t-test statistics (Steel and Torrie, 1960) were used for comparing the geometric means of bacterial counts.

Table 1: The LFC and ECC of bacteria in the food samples

		No of +ve samples (%)		GMC (Log_{10} CFU mL^{-1} or g)	
Food type	Frequency (%)	LFC	ECC	LFC	ECC
Beans	9 (11.84)	7 (77.8)	2 (22.2)	8.41	6.46
Yam	9 (11.84)	6 (66.7)	4 (44.4)	8.35	6.93
Abacha	6 (7.89)	5 (83.3)	5 (83.3)	9.26	7.81
Okpa	6 (7.89)	4 (66.7)	3 (50.0)	8.87	7.48
Moi-moi	6 (7.89)	4 (66.7)	4 (66.7)	8.65	7.41
Pear	6 (7.89)	6 (100.0)	6 (100.0)	9.21	6.34
Cassava (foofoo)	6 (7.89)	2 (33.3)	1(16.7)	7.64	6.50
Rice	6 (7.89)	3 (50.0)	3 (50.0)	8.75	6.54
Agidi	6 (7.89)	6 (100.0)	5 (83.3)	8.64	7.40
Garri	6 (7.89)	4 (66.7)	4 (66.7)	8.65	7.53
Water	10 (13.16)	8 (80.0)	8 (80.0)	5.32	4.46

RESULTS AND DISCUSSION

About 76 samples were examined in the study where made up of 66 food samples and 10 water samples. Out of the 66 food samples, beans and yam were 9 samples each while the rest of the samples (abacha, moi-moi, okpa, rice, agidi, garri, cassava foofoo and pear) were 6 samples each.

About 2 major methods, the Most Probable Number (MPN) and the Plate Count (the lactose Fermentation Count (LFC) and *E. coli* Count (ECC) were used. Counting the pink coloured colonies of lactose fermenter on MCA or counting the colonies with green metallic sheen representing *E. coli* colonies on EMBA determined the coliform load. Most of the food samples were heavily contaminated. The Geometric Mean Count (GMC) in the foods for total count or lactose fermentation count ranged from 7.64-9.21 while that of *E. coli* count ranged from 6.34-7.81 (Table 1).

The proportion of the various food types contaminated sometimes varied according to the technique used in coliform detection and enumeration. For example, in LFC 77.8% of beans samples were contaminated while the ECC gave 22.2% contamination for the same food sample. However, for some food types, the proportion contaminated were the same for the different enumeration techniques. Example, in both the LFC and ECC 66.7% of moi-moi and 50% of rice were contaminated (Table 1). The MPN count ranged from 0-180 (Table 2). A

Table 2: The MPN count of bacteria in the food samples

	Most Probable Number (MPN)		
Food type	0-10	11-50	51-180
Beans	3	-	6
Yam	3	1	5
Abacha	-	-	6
Okpa	2	1	3
Moi-moi	2	-	4
Pear	-	-	6
Cassava (foofoo)	4	1	1
Rice	3	-	3
Agidi	2	-	4
Garri	3	-	3
Water	2	-	8

Table 3: Percentage of positive food samples in the different enumeration methods

	Isolation methods		
Food type	MPN (%)	LFC (%)	ECC (%)
Beans	67.2	77.8	22.2
Yam	57.0	66.7	44.4
Abacha	92.0	83.3	83.3
Okpa	42.0	66.7	350.0
Moi-moi	67.0	66.7	66.7
Pear	96.3	100.0	100.0
Cassava (foofoo)	20.0	33.3	16.7
Rice	48.1	50.0	50.0
Agidi	67.0	100.0	83.3
Garri	50.0	66.7	50.0
Water	92.4	80.0	80.0

Table 4: Bacterial count according to condition of food

	GMC (Log_{10} CFU mL^{-1} or g^{-1})	
Food condition	LFC	ECC
Freshly prepared/cooked	7.89	6.77
Served in plates	8.72	7.50

Table 5: Percentage positive samples in relation to storage and duration using MPN count

Food type	Freshly prepared/ cooked	Served in plates
Beans	3.9	100.0
Yam	10.0	100.0
Abacha	100.0	100.0
Okpa	0.0	10.0
Moi-moi	0.0	100.0
Pear	88.9	100.0
Cassava (foofoo)	0.0	1.7
Rice	0.0	100.0
Agidi	0.0	100.0
Garri	0.0	0.0
Frequency of positive sample (%)	40.0	90.0

comparisons of the three methods of isolation using the percentage of positive samples is shown in Table 3. There was no significant difference ($p>0.05$) between the three enumeration methods used however significant difference ($p<0.05$) in coliform count exists amongst the various food types.

The GMC of food obtained directly from the pot or immediately after preparation and that obtained from service plates for both the LFC and ECC is shown in Table 4 while that of MPN count is shown in Table 5. A total of 98 isolates were obtained from these samples, 45% of these isolates were identified as *Klebsiella pneumonia* and 51% identified as *E. coli*, the remaining 4% were suspected to be Enterobacter. Of the 98 isolates, 20 (20.4%) were suspected to be of non-human origin because they failed to grow at 44°C while the remaining 78 (79.6%) which showed growth at 44°C were of human origin (Table 6). This research present a study on the biological quality of food and water consumed in Nsukka area. The basic aim being to determine the level of personal and environmental hygiene in the area as indicated by the level of contamination in these food samples examined. These food samples were obtained ready for consumption from vendors and stewards in restaurants. The water samples collected along side with the food samples were those presented for drinking or used in washing utensils used in the serving the food. The major aim of screening water samples was to determine the probable role in contamination of food or direct infection of consumers.

Coliforms in general and *E. coli* in particular are widely referred to as indicators of feacal contamination and their acceptable count in food in developed countries is 10^4 CFU g^{-1} (Cooke and Gibson, 1990). The two major methods (MPN and Plate count methods) used in the enumeration of coliforms, the counts obtained were by

Table 6: Bacterial isolates from samples

Isolates	Frequency	Human origin	Percentage of human origin
Klebsiella pneumonia	44.0	37	47.4
E. coli	50.0	41	52.6
Enterobacter	4.0	-	-
Total	98.0	78	

far higher than the above mentioned standard. Using three different techniques ensured higher chances of detecting false positives and false negatives. Besides, the media used both for the MPN and LFC techniques contained bile salts which suppresses growth of other microorganisms other than lactose fermenters. In the case of the ECC technique, the green metallic sheen appearance of *E. coli* colonies is unmistakable. The result obtained from the MPN and LFC have shown a higher degree of agreement and this would be expected since both techniques gave the total count of the coliforms present. The counts for the LFC and ECC sometimes varied. This is not surprising as the *E. coli* is only one of the coliforms counted in the LFC method. In a few cases however, the counts were similar. For these cases however, even the organisms isolated were identical. Statistically there were no significant difference ($p>0.05$) in the three methods.

Coliforms isolated from food in various places are enough reason for concern and more so when present in food in such high numbers become risks of gastroenteritis and particularly diarrhoea. The high level of food contamination observed in this study had also been reported earlier in Nigeria and other developing countries including Senegal, Bangladesh and Ghana (Tomkins, 1981; Iroegbu *et al*., 2000; Al-Khatib *et al*., 2004; Ifediora *et al*., 2006; Yeboah-Manu *et al*., 2010). All these regions are common for their poverty and low standard of personal hygiene and environmental sanitation.

According to Cheesbrough (1993) the suggested bacteriological criteria for drinking water from unchlorinated rural hand pumps and others (also adapted for food samples) for the MPN is that all counts at 0 are excellent while counts between 1 and 10 are acceptable, counts from 50 and above are grossly polluted and therefore highly unacceptable and unsuitable for consumption. In this study the coliforms occurred in greater numbers in the unacceptable category (Table 2). The plate counts also indicated unacceptably high bacterial colony counts. In recent times, there has been a constant report of out breaks of gastrointestinal tract infection in several parts of the country and these were blamed according to the nature of the food types especially beans and vegetable and the method of preparation. Coliforms belong to this group of bacteria that do not resist heat and so their rate of occurrence in

the examined samples seems to be greater in those food types that do not require heat in preparation as in the case of pear and abacha (Table 5). About 80% of food samples collected directly from the pot immediately after cooking showed little or no coliform contamination while the remaining 20% of the samples collected immediately after preparation that showed positive results are those that do not require boiling/cooking or any form of heat during preparation (pear and abacha). For the 80% that showed no contamination after preparation, some turned out to be greatly contaminated after they have been served out in plates which were washed with water from the available water source. This point to external contamination resulting from either contaminated water or from poor hygiene.

Merlin (1969); WHO (1993) and Mensah (1997) indicated that high concentration of coliforms in food could lead to such symptoms as nausea, vomiting, retching, abdominal cramp, diarrhoea and prostraction. Thus the high concentration of coliforms found in this research could constitute a health hazard without presence of actual enteropathogenic strains.

Most families in Nsukka do not have access to good water supply. The taps run intermittently and only a few household have access to them. Therefore, most households wander all day in search of water and resort to collecting from drainage gutters or broken water supply pipes. Others buy from water tankers whose sources include streams. Some others collect from ponds. All these sources are likely to be contaminated from the environment especially during flood. In Nsukka also, like in most rural communities most households do not have good toilet facilities and so they defeacate indiscriminately in the surrounding bushes. They may be washed by flood to the above water sources and hence the predominance of human coliforms in the water samples.

Another source of contamination was noted and this is poor personal and environmental hygiene. This is shown as in the case of moi-moi where the water source is free from of coliform and the sample collected directly from the pot immediately after cooking was also free from contamination while that collected during serving was heavily contaminated. This shows that the environment and handlers contaminated the food. In unhygienic environments flies perch on dirt and then on food or on plates which are ready to be used for serving. Poor personal hygiene of handlers could be as a result of using unwashed hands to handle food or utensils especially after making use of the toilet. Majority of the coliforms isolated were of human origin while a negligible number were of non-human origin. Probably, those of human origin resulted from feacal contamination of food and water. The coliforrms of human origin isolated from food samples were identified as *Klebsiella pneumonia* and *Escherichia coli*. Although *E. coli* is a normal inhabitant of the human gut, several serotypes have been known to cause diarrhea in infants as well as adults (Merlin, 1969; Ifediora *et al*., 2006). Thus detection of human *E. coli* strains in food and water presented for human consumption signals a health hazard even though the strains may not be specifically identified as enteropathogenic.

CONCULSION

In conclusion, three major sources of contamination have been identified and these include water, method of food preparation as in the case of abacha where heat is not applied and fermented spices (ogiri) are used and poor environmental and/or personal hygiene of handlers.

Thus, there is the need for establishing strict health rules for food vendours and restaurants in Nsukka. There is also need for regular check on the microbiological quality of food and water consumed in the area. These will go a long way to reducing the frequency of occurrence of gastroenteritis in the locality.

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