Estimation and Characterization of Coliforms in Vended Food and Water Samples in Nsukka Area

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Abstract

Sixty–six (66) food and ten (10) water samples from Nsukka metropolis were screened for the presence of coliforms using the Most probable number (MPN) technique and direct plate count on MacConkey agar/broth and Eosin Methylene Blue (EMB) agar for isolating those from both faecal and human origin. The isolates were characterized biochemically analysed using standard conventional methods. The geometric mean counts (GMC) using MPN ranged from 1-180 faecal coliforms per 100 ml for both food and water samples. However, food samples (66.7%) served in plates and water sources (83.3%) showed apparent signs of contamination probably from the human and water sources, handling and storage unlike foods from the pots that had no trace of contamination. Faecal coliforms were E. coli (42.4%) and Klebsiella pneumonia (37.4%) and others(20.20%)of non-human origin. The E. coli count ranged between 22.2% ("abacha") and 83.3% (beans ,rice, yam) as well as water(50.0%).Thus, there was no significant difference (p>0.05) between the indicator organisms (E. coli) in the food, storage vats and water sources. The faecal and human coliforms in foods and water were indications of foodborne diseases

Keywords: Food, Water, Coliforms, Vending, Contamination

Introduction

Food safety is a growing concern for consumers and professionals in the food and food service industry (Scheule and Snead, 2001).Food safety is defined as the conditions and measures that are necessary during production, processing, storage, distributions and preparations of food to ensure that it is safe, sound, wholesome and fit for human consumption (WHO,1984).The WHO food safety unit has given high priority to the research of preservation as a technique for preparation /storage of food. Efforts are geared towards the assurance of food safety as a guard against food borne illness. According to WHO (2002), food borne diseases are reported to be widespread in the contemporary world and are responsible for about one third of deaths worldwide. Many microorganisms have been found to proliferate readily at a wide range of temperatures (Egonu and Allan, 2000). Some bacteria that are important from public health of view may multiply to dangerous high levels in food without changing their appearance, odour and taste (Nwabueze and Archibald, 1997). Many human diseases are acquired from contaminated drinking water and food preparation. To assure safety, municipal water supplies are routinely tested (Dibua *et al*., 2007). It would be impracticable to test. According to Okaka and Ene (2005), the common indicator group of organism for food and water contamination are the coliform bacteria and these coliforms refer to member of the family Enterobacteriaceae include the genera *Escherichia* and *Aerobacter (*Jay, 2004*).* However, the presence of coliforms does not always indicate fecal pollution (Adam and Moss, 1999). It is important to differentiate the type of the coliform in order to certify whether a sample of water in contaminated from a fecal source or not. The monitoring and detection of indicator and disease-causing microorganisms are a major part of sanitary microbiology (Frazier and Westhoff, 2005; Prescott *et al.,* 2005).

Coliform bacteria are defined as aerobic and facultative anaerobic, Gram-negative non-spore forming, rod-shaped bacteria that ferment lactose such as *Escherichia coli* which is almost always present in faeces of humans and other animals (Ingraham and Ingraham, 1995; Prescott, *et al.,* 2005). In the United States, a set of general guidelines for monitoring microbiological quality of drinking water had been developed including standards for coliforms, viruses and *Glardia (*Prescott *et al.,* 2005). Such promulgated regulations do not exist for developing countries like Nigeria among others. The greater percentage of morbidity and mortality in developing countries are linked to water-borne infectious diseases and these water-borne diseases have been occurring in many families in Nsukka metropolis but proper attention had not adequately been given to the origin of such diseases. Meanwhile, the public health significance of some sources of water used for drinking and food preparation in Nsukka is questionable and serve as a delivery route for high level of contamination. There is the need for bacteriological survey in Nsukka just as was conducted in Idah, Kogi State of Nigeria (Peters and Odeyemi, 1990; Abu *et al.,* 2006).

Food has also been implicated as a possible source of infectious organisms, when contaminated

(Okaka and Ene, 2005). In Nsukka, some foods are vended by local hawkers and such foods are hawked at medium and small-scale especially in cafeteria, restaurant and hotels or even in wheel barrows and open trays/pans. The vending of food seems to be a source of livelihood for people especially women and for convenience among students as well as career/working class women who do not have ample time for food processing. Some of the indigenous foods vended in and around Nsukka metropolis include the indigenous diets based on popular staples such as yam (*Dioscorea* species*),* Cassava (*Manihot esculenta*) (Eka, 1998), Maize (*Zea mays*) (NAS, 1979; Okoh, 1998), Plantain (*Musa* species) and legumes of various kinds (Okeke and Ene-Obong, 1995; Elegbede, 1998).

Since water is used in various stages of processing, handling, and storage of these vended foods, the quality of the water would in turn affect the quality of the food. This study aimed at estimating the occurrence of coliforms in food samples and water samples used for food preparation from various eating places in Nsukka metropolis.

Materials and Methods

Sixty-six samples of different foods were collected from different food vendors and 10 water samples used for the food preparation were collected alongside with the respective foods. Thus, a total of 76 samples were obtained from eating places in Nsukka market and within the University of Nigeria, Nsukka campus. The food types were six samples of "agidi", six samples of "okpa", six samples of "moi-moi", six samples of rice, nine samples of yam, nine samples of beans, six samples of "garri", six samples of cassava "foo-foo", six samples of "abacha" and six samples of pears. All those foods collected were in the state for immediate consumption. Some of the samples were collected wrapped in leaves or polythene or contained in tins/cans; served in plates, basins or pots. Each sample was collected in a fresh sterile polythene bag using a sterile spoon. All samples were transported to the laboratory and analysed within 3 hours of collection.

Media used: The media used for the analyses of the samples were compounded according to the manufacturers' directions (Oxoid, 1982). These media included MacConkey broth, peptone water sugar, urea agar base and Simmons citrate agar. Heat-sensitive media were sterilized by autoclaving at 121^0C and 15 pounds for 15 minutes or at 115^0C and 10 pounds for 10 minutes. After sterilization of the media, they were dispensed aseptically into sterile Petri dishes and universal bijou bottles for slants as appropriate. They were incubated overnight at 37^0C for sterility checks before use.

Estimation of coliforms: Coliform count was done using most probable number (MPN) technique. Three sets of tubes were used per dilution. In the first set of tubes 10 millilitres of samples was incubated with 10 millilitres of double strength lactose broth. In the second set of tubes, 1 millilitre of sample was inoculated with 10 millilitre single strength lactose broth. Durham tubes were inserted and inverted in all the tubes. The tubes were covered and incubated at 37^0C for 24 hours. Tubes that showed gas production or effervescence after 24 hours incubation were noted as positive tubes. Tubes that appeared negative were reincubated for another 24 hours. All positive tubes were charted and the MPN table was consulted. Positive tubes were further subcultured into on lactose broth and incubated in a water bath at 44^0C, 24 hours and onto nutrient agar slant and incubated at 37^0C and 24 hours. Production of gas and indole at 44^0C showed the stained to the presence of .faecal *E. coli*. Growth on the nutrient agar slant was gram stained to confirm the presence of the coliforms.

Purification of isolates: The total coliform load of the food samples was determined by the examination of the colonies in MacConkey agar while total *Escherichia coli* load in the food sample was determined by enumeration in colony forming unit in EMB- agar. Colonies developing on EMB and MacConkey agar plates were subcultured on fresh nutrient agar (NA) plates and incubated at 37^0C for 24 hours. Pure isolates were stocked on nutrient agar slants, which were maintained at 4^0C.

Identification of coliforms of human fecal origin (growth at 44^0C): Colonies from MacConkey and EMB agar plates were characterized based on colonial morphology, Gram stain and various biochemical reactions according to standard microbiogical technique (Harrigan and McCance, 1976; Collins and Lyne, 1979; Henry *et al.,* 1987; Speck, 1988; Cheesbrough, 1993).

The sterile MacConkey broth was used here. A loopful of the isolates was inoculated into the broth in tubes containing inverted Durham's tubes. Then, the tubes were incubated at 44^0C for 24 hours. Changes in colour from purples to yellow indicate acid production. The presence of gaseous or air bubbles in inverted Durham tubes show gas production.

Results

Most probable numbers: The geometric mean counts (GMC) ranged from1-180 faecal coliforms per 100ml. The GMC for various food and water samples shown in Table1. For the *Escherichia coli* counts, the proportions of various foods with apparent contamination were as follows: beans (22.2%), yam (44.4%), "okpa" (50.0%), rice (50.0%), garri (50.0%), cassava foo-foo (16.7%), moi-moi (66.7%), agidi (83.3%), "abacha" (83.3%) and pear (83.3%). There was a significant different Table 2 between *Escherichia coli* count and the total coliform count.

Food obtained directly from pots after cooking did not show any notable contamination while 66.7% of duplicate samples of same foods served in plates but

Table 1: Positive samples in relation to food group (type) using the most probable number method

Food sample	Number of Positive Tubes/MPN Value		
	0-10	11-50	51-180
Beans	5	6	4
Yam	3	-	6
"Abacha"	-	-	6
"Okpa"	2	1	3
"Moi-moi"	2	-	4
Pears	1	-	5
Cassava "foo-foo"	4	1	1
Rice	3	-	3
"Agidi"	2	-	4
Garri	3	-	3
Water	2	-	8

Key: *Moi-moi = steamed cowpea paste; "Okpa" = Steamed bambara groundnut paste; "Agidi" = Maize meal; "Abacha" = African cassava salad; "Garri = fermented, fried cassava meal; cassava foo-foo = Fermented cassava meal.*

Table 2: Coliform and *Escherichia coli* contamination (cfu/g) in relation to food group (type)

Food Type	Number examined	No. positive for *Escherichia coli*	%	Total no. positive for coliform	%
"Abacha"	6	5($6.42x10^7$)	83.3	5($1.81x10^9$)	83.3
"Agidi"	6	5($2.50x10^7$)	83.3	6($4.37x10^8$)	100.0
Bean	9	2($2.89x10^6$)	22.2	5($1.02x10^8$)	55.6
"Cassava foo-foo"	6	1($3.17x10^6$)	16.7	2($4.33x10^7$)	33.3
"Garri"	6	4($3.42x10^7$)	50.0	4($4.42x10^8$)	66.7
"Moi-moi'	6	3($2.58x10^7$)	66.7	4($4.33x10^8$)	66.7
"Okpa"	6	3($3.02x10^7$)	50.0	4($7.48x10^8$)	66.7
Pear	6	5($1.75x10^7$)	83.3	5($1.28x10^8$)	83.3
Rice	6	3($3.50x10^6$)	50.0	3($5.58x10^8$)	50.0
Yam	9	4($8.44x10^6$)	44.4	6($2.27x10^8$)	66.7
Water	10	8($2.07x10^5$)	50.0	8($3.01x10^4$)	80.0

Key: *( ) indicates the average colony counts in Cfu/g for broth Escherichia coli and the coliform bacteria*

Table 3: Levels of contamination of the food sample using different modes of storage

Food sample	Pots	Plates	Water
Beans (B)	0	1	1
Yam (Y)	0	1	1
"Abacha" (A)	-	-	-
"Okpa" (O)	0	1	1
"Moi-moi" (M)	-	-	-
Pears (P)	-	-	-
Cassava "foo-foo" (CF)	0	0	1
Rice (R)	0	1	1
"Agidi" (AG)	-	-	-
Garri (G)	0	0	0

Key: *1 = Positive; 0 = Negative and - = Not analyzed*

washed with available water sourced were contaminated. Approximately 83.3% of these water sources were also contaminated with faecal coliforms as shown in Table 3. There were no significant differences ($p<0.05$) in the level of contamination of individual food types and the modes of storage.

The frequency distribution of the positive contaminated food samples is shown in Table 4 using both storage devices (plates and pots) as well as the water used for the food preparation and washing of the cooking utensils and serving the foods. The correlation of *Escherichia coli* contamination (based on the number of the positive samples) in relating to the method of storage and water used in serving the food showed that there was no significant difference ($p>0.05$) between the *Escherichia coli* count and the storage rates as well as the water sources.

Coliforms: Of all the bacterial colonies isolated, 42.4% were identified as *Escherichia coli*, 37.4% as *Klebsiella pneumoniae* and the rest unidentified were designated merely as coliforms as shown in Table 5. The identified isolates were all human origin while most of the unidentified isolates were of non-human origin (20:20%) as shown in the Table 5.

Biochemical characteristics of the isolates: The biochemical characteristics of the bacterial isolates are shown in Table 6.

Discussion

The Most probable number (MPN) plating technique was used for the estimation of the level of microbial contamination of the foods studied. Both the foods and water samples were highly contaminated and this is hazardous with high risk of diarrhoea. The acceptable limit of coliforms in foods with developed countries is 10^4 colony forming unit per gram (Cooke and Gibson, 1990). Meanwhile, the bacterial counts obtained in this study were far higher than the above standard as shown in Table 2.

The high level of contamination of foods, observed in this study had also been reported in some developing countries such as Ethiopia (Jawa *et al.,* 1981), Gambia, Senegal and Bangladesh (Beau *et al.,* 1987; Chen *et al*., 1981; Rowland *et al.*, 1978); Tajikistan (Mermin *et al*., 1999) and Nigeria (Iroegbu *et al.,* 1994). The common features of these regions were their underdevelopment, poverty rate, low standard of personal hygiene and environment sanitation.

Also, two types of coliforms were identified in the study which were the human and non-human coliforms. It appeared that human coliforms were from faecal source while the non-human coliforms arose from contact of items with non-faecal environmental contaminants. The existence of a natural coliform flora of fresh water is a matter of divided opinions as Henriksen (1954) reported that for a specific aquatic flora of coliforms among strains that were more frequently found in water than in samples of faeces. He suggested that the enteric organisms and urease-negative *Aerobacter* strains constituted such a group.

Table 4: Frequency distribution table of positive food samples

Sample	Total number of Samples	Number of positive samples	Percentage of the positive samples
Foods (in pots)	6	0	0.0
Foods (in plates)	6	4	66.67
Water	6	5	83.33

Table 5: Frequency of the Isolates

Isolates	Frequency of occurrence	% Frequency
A	37	37.37
B	42	42.43
C	20	20.20
Total	90	100.00

Where; A = Klebsiella pneumoniae; B = Escherichia coli; C = Non-human coliform bacteria

Henriksen (1954) concluded that about third-quarter of the coliforms in water might be of faecal origin.

The faecal coliforms of human origin isolated from the contaminated food and water samples were *Klebsiella pneumonia* and *Escherichia coli. Klebsiella pneumoniae* could be found in the respiratory tract and faeces of normal individuals (Brooks *et al.,* 2004). Thus apart from the faecal source, the respiratory tract could be a source of contamination of *Klebsiella pneumoniae.* The common features of both coliforms identified are that they have affinity for the gastro-intestinal tract (Feng and Hartman, 1982).

In this study *Escherichia coli* isolated had the highest frequency (42.42%). Its presence in these foods indicates a diarrhoeal risk for consumers of the contaminated food or risk of gastro-enteritis in general. The presence of *Escherichia coli* in food and water certainly show low standards of personal and environmental hygiene. It was noted that 18 out of about 150 distinct serological types are implicated in diarrhoea in man. There lies the public health significance of the observation in this study. *Klebsiella pneumoniae* occurred less frequently than *Escherichia coli* at a frequency of 37.3%. It rarely causes diarrhoea but could liberate enterotoxins under disease conditions. Thus, it has a public health implication (Ventateswarean *et al.,* 1996).

The non-human faecal coliforms were isolated but were not characterized for proper identification. The percentage frequency of occurrence of these organisms was the lowest (20.20%). It was surprising that *Enterobacter aerogenes was* not implicated in these gross food contaminations. The reason is not clear. However, due to its close resemblance to *Klebsiella pneumoniae* on MacConkey agar, it might be that it was mistakenly left out during preliminary isolation.

Modes of storage were also examined to trace the possible sources of contamination of foods. Some were preserved in the pots while others were served in plates. The foods stored in pots were found not to be contaminated. After elimination of organisms by heat during cooking in the pot, covering the pot additionally ensured that contaminants were not seeded by flies or air current. Though some food vendors claimed to have prepared the foods early in the morning of sale, contamination still resulted in those foods served in plates. This may have arisen from the use of contaminated water in washing of the plates and utensils (used items). However, water was usually stored in a holding tank in which any container was observed to be freely dipped inside all the time without adequate control and care for the health status of the consumers. Through this practice, a potential hazardous pathogen could be introduced to the water which eventually are served to the consumers of such food.

Sadiq and Abdullahi (2008) reported that the poor hygienic conditions of the food handlers made them obviously unclean. Adesiyun and Kwaya (1983) identified the cafeteria environment and their workers as likely sources of food contamination. Okolocha and Ayide (2006) reported that persons involved in the food preparation, the water used may serve as sources of contamination. Likely other sources of contamination might include the handlers' body water for food preparation and the general environment. The unhygienic state of the food hawkers' handling might lead to introduction of the microbes into cooked foods. Since the water for drinking is cool, its temperature is suitable for the existence of microbial forms unlike water for cooking in which any contaminating micro-organism could be eliminated during cooking.

It is obvious that the presence of coliform bacteria in food is undesirable. As most contamination is likely to occur in the process of handling or cooking, storage should be in appropriate clean containers. Thorough warming of stored foods to at least 70^0C would reduce their coliform bacterial load and thus, eliminate pathogens (Cooke and Gibson, 1990). The effectiveness however depends on the length of time it is heated. If the time is relatively short, then there will be no time for the heat to penetrate and destroy the already multiplied microbes. Too much heat may destroy certain nutrients in the food. Therefore, a compromise heat level such as the one used in pasteurization should be applied to penetrate and destroy most bacterial cells without altering the food quality.

Furthermore, an integrated approach is needed to prevent food-borne disease by improving on the environmental conditions; through the provision of portable water supply, proper sanitation and socio-infrastructural facilities to help the hawkers to improve on their hygiene. Health education in food safety should receive priority, considering that food contaminated by improper handling is responsible for a greater proportion of food-borne diseases. Appropriate food supply educational programs that are well adapted to the socio-cultural condition of the target groups should be designed and applied to ensure good manufacturing practices (GMP) during

Table 6: Biochemical characteristics of isolated coliforms in vended food and water samples in Nsukka area

Colony morphology	Gram stain rxn	Growth at 44^0C	Indole	Oxidase	Citrate	Urea	Lactose	Mannitol	Glucose	Maltose	Sucrose	Slope	Butt	H_2S	Gas	Probable organisms	Isolates
Pinkish, circular raised, mucod colonies with entire margin and size between 2-5mm	Gram negative short, stout rods	+	-	-	+	+	AG	AG	AG	AG	AG	Y	Y	-	G^1	*Klebsiella pneumoniae*	Ag1 A; Ag2 A; Ag3 A ; Ag 5i A; Ag 5iiA; Cf2A; G1A; A2A; PIA; P5iA; AIA; A2A' A3A; A4A; MIA; M5iiA; RWA; B1A; B2A; B5A; R8iiA; BWA; YIA; Y3A; Y4A' Y8i a; Y8iiA' YWA; 01A; 02A; 04A; OWA
Pinkish, circular, soft, flat, flat colonies with entire margin and size between 2-4 mm	Gram negative fairly long rod	+	+	-	+	+	AG	AG	AG	AG	AG	Y	Y	-	G^3	*Escherichia coli*	Ag_2B; Ag_3B; Ag $5ii_B$; AgwB; Cf_2B; Cf_3B; CfwB; G_1B' G_2B; G_3B; P_1B; P_2B; P_3B; P5iB; PwB; A_1B; A_2B; A_3B A_4B; A5iB; A5iiB; AwB; M_1B; M_3B; M_4B; M5iiB; R_5B; R_4B; B5iibB; RwB; B_5B; B8iiB; BwB; Y_2B; Y_3B; Y8iB YbiiB; YwB; O_1B; O_4B; O5iiB; OWB;
Reddish, circular, soft colonies with entire margin and size of 2-3mm	Gram negative short rods	-	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Non-human coliform	Ag_4A; P_4A; M_3A; R_1A; B_3A; B_4A; A_3B; M_1B; AgB; Ag_4B; Ag_4B; g_4B; P_4B; R_1B; B_1B; Y_4B; M_1C; R_3C; B_2C; B_3C

Note: *AG = Acid and Gas production (±); (+); - Positive test; (-) Negative test; A = Acid production; Nt- Not tested, Ag = Agidi; A = Abacha; B =Beans; C = Cassava foo-foo; G = Garri; R = Rice; P = Pears; M = Moi-moi; O = Okpa; Y = Yam; W = water*

manufacturing, packaging or holding of human foods. These codes of practice should cover plant and grounds, equipment and utensil, sanitary facilities and control, sanitary operations, processes and controls as well as personnel. Also, there should be specific good manufacturing practices written for African indigenous foods just as there was some proposed GMPs and seafood products, cacao products and confectionary, bakery food, bottled water, tree nuts and peanuts, pickled, fermented, acidified and low-acid foods as well as thermally processed low acid foods package in hermetically scaled containers to assure food safety and wholesomeness during vending.

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