Incidence of Faecal coliforms Isolated From Different Foods Sold Locally in Nsukka, Enugu State

[1]Mbaeyi, I.E.* and [2]Iroegbu, C.U.
[1]Department of Food Science and Technology, University of Nigeria, Nsukka
[2]Department of Microbiology, University of Nigeria, Nsukka.

Corresponding Author: Mbaeyi, I.E.; Phone: +2348185143920; +2348037722818
Email: miphie2003@yahoo.co.uk or ifeoma.mbaeyi-nwaoha@unn.edu.ng

Abstract
Sixty-six (66) food products and ten water samples were collected from food vendors and restaurants and examined for bacteriological quality using both the most probable number (MPN) technique and the plate count of Escherichia coli on Eosin Ethylene Blue (EMB) and lactose fermenters on MacConkey agar. All results obtained were analyzed using ANOVA and Duncan's multiple range test. Out of the 66 food samples, 55.6% were positive for Escherichia coli as follows: beans (22.6%), Yam (44.4%), garri (50%) pears (83.3%), cassava "foo-foo" (16.7%), "abacha" (83.3%), "okpa" (56%), rice (56%), "agidi" (83.3%) and moi-moi (66.7%). Out of the ten water samples, six were also positive for Escherichia coli. Escherichia coli high proportion of food (75.9%) and water (80%) respectively showed the presence of coliforms in general including Escherichia coli Geometric mean count (GMC) for MPN ranged from 1-180 and GMC for coliforms in various foods ranged from 1.0 x 10^1-1.0 x 10^6cfu/g. About 42.4% human coliforms(Escherichia coli), 37.4% of faecal coliforms (Klebsiella pneumonia) and rest (20.2%) showed other coliforms in food and water samples.

Keyword: Food, Water, Coliforms, Most Probable Number, Geometric Mean Count

Introduction

Food is one of the basic needs of man. It gives and provides energy for different activities of life. For instance, most foods are complex mixtures of organic and inorganic chemical compounds which the body requires to grow and maintain itself in healthy condition (Enwere, 1998). According to Davidson *et al.* (1975), it is utilization for body building growth, maintenance and repairs of living tissues and for the provision of protection against diseases through the regulation of body metabolism. These functions could be performed by the six classes of food. While some people have the facilities and can spare the time to prepare food in their houses, many others would patronize food vendors in open market places and restaurants. If the importance of a food nutrient is judged by how long we can do without it, water ranks as the most important (Dosumu *et al*., 2009).

Food hygiene is essentially aimed at producing food which is safe for human consumption and of good keeping quality (Adesiyun, 1984). The hygiene standard of a food is based on good manufacturing practice as well as the conditions of the raw materials. A food item prepared from water contaminated with pathogenic microorganisms will successively be contaminated and a health risk. Byran (1988) identified the most common food handling mistakes to include serving contaminated raw food materials, inadequate cooking or reheating of cooked foods, obtaining food from unsafe sources; cooking foods inappropriately; allowing too much of time lapse between cooking and consumption. Certain processes or handling practices in food preparation (like washing of hands before food handling and serving food hot) have been identified as being essential or critical in preventing food borne disease (Altekruse *et al*., 1995). However, these practices are not fully abided by the food vendors. Similar reports were identified by Adesiyun and Kwaya (1983) indicating that cafeteria staff and other food service workers are likely sources of food contamination.

In Nsukka area, foods sold by vendors and in restaurants include rice, beans, yam, pears, cassava, foo-foo, garri, "abacha", "okpa", moi-moi and "agidi". They are available at the consumer's convenience and offered as ready-to-be-taken foods. These foods are consumed by many people and in large quantities. The foods are sometimes poorly stored after cooking and therefore, susceptible to microbial contamination and spoilage as substrates for microbial growth (Jay, 2004; Frazier and Westhoff, 2005; Okaka and Ene, 2005). Frequent food contamination results with the introduction of pathogens from unwashed hands, fomites or indeed contaminated water used for washing.

According to WHO (1993), water is also considered an essential element of food. Thus, both foods and water can be important vehicles for transmission of certain enteric pathogens giving rise to food and water-borne diseases (Adam and Moss, 1996) such as diarrhoeal infections (Jiwa *et al.,* 1981). Route of entry of such pathogen is invariably the oral route. It is not often practicable to search for a pathogen in a large quantity of food or water. The detection and enumeration of indicator bacteria are of primary importance for monitoring sanitation and microbiological quantity of food and water (Feng and Hartman, 1982). The possibility of pathogen being present in food and water is often

inferred from the presence of faecal coliforms. Coliforms and faecal coliforms(*Escherichia coli*) are all used as indicators of faecal pollution according to Brooks *et al.* (2004). Among these *Escherichia coli* is often preferred as an indicator because it is specific and most reliably reflects faecal origin.

By virtue of definition, coliforms are gram-negative, oxidase-negative, non-sporing rods that are capable of fermenting lactose to produce acid and gas within 24 hours at 37^0C (Prescott *et al.,* 2005). It is possible to distinguish faecal coliforms from those of plant origin because faecal coliforms are able to ferment sugars at 44^0C. The microbes used as indices include *Streptococcus faecalis, Klebsiella, Enterobacter* and *Citrobacter* species, apart from *Escherichia* (Clark and Pagel, 1997). Thus, as indices of faecal contamination, they are used in the determination of the sanitary quality of food and water.

Food and water are found to be highly contaminated with coliform or other indicator bacteria suggest risk of gastroenteritis, particularly diarrhoea which accounts for high rate of morbidity and mortality especially in under-developed countries of the world (Jiwa *et al.,* 1981; Mermin *et al.,* 1999). Microbial pollutants also cause a widespread incidence of food poisoning. Pollution is concomitant with low standard of hygiene in certain communities. Nigerian communities being inclusive in the above representing a good example of places where food served in public eating-places and from vendors could be highly contaminated and possibly contain pathogens. Also, in countries where street vending of food is prevalent, there is commonly a lack of information on the incidence of food borne diseases related to the street vended foods (Mahale *et al.,* 2008) .

This work was to determine the bacteriological quality of food and water sold by vendors and restaurants and mainly consumed by common people in Nsukka, Nigeria. Attempts were made also to
identify the contaminants in order to ascertain the presence or absence of pathogens in the sample. Coliforms (*Escherichia coli)* in general are used as indicator organisms for faecal contamination of food and water.

Materials and Methods

Sample collection/preliminary analyses: Sixty six (66) samples of different foods were collected from different food vendors and 10 water samples used for food preparation were got alongside with the respective foods. All the samples from the different sources were microbiologically examined using two assay methods [most probable number (MPN) technique and direct plate count enimeration] for the coliform colonies in appropriate media. A total of 76 samples were obtained from eating- places in Nsukka market and from within the University of Nigeria, Nsukka Campus. The food types were “agidi”(6), “okpa”(6), moi-moi, rice(6), yam(9), beans(9), garri (6), cassava foo-foo (6), “abacha”(6) and pears (6). All foods collected were in the state for immediate consumption. Some of the samples were collected wrapped in polythene or contained in tins/cans, or served in plates, basins or pots. Each sample was collected in fresh sterile polythene bags using sterile spoon. The samples were put into pre-sterilized conical flasks or test tubes and covered with aluminum foil before bacteriological examination. All samples obtained were taken to the laboratory within 3 hours and maintained at about 4^oC prior to analyses.

Preparation of media: The media used for the analyses of the samples were compounded according to the manufacturer’s prescription (Oxoid Manual, 1982). The media were MacConkey broth, MacConkey agar, Nutrient agar, Eosin methylene blue (EMB) agar, Glucose phosphate peptone water, Peptone water, Urea agar base and Simon citrate agar (Oxoid Ltd., Basing-stoke England). All the heat-sensitive media were sterilized by autoclaving at 121^0C and 15lbpsi for 15minutes or at 115^0C and 10lbpsi for 10 minutes. After sterilization of the media, they were dispensed especially into sterile petri dishes and universal bijou bottles for slants as appropriate. The media were incubated at 37^oC for 24 hours and checked for sterility.

Estimation of coliform count by mpn technique: MPN technique was used for the detection of faecal coliforms in the samples using the MacConkey broth having different amount, strength and number of Durham tubes (APHA, 1998). A known weight, 25g of each of the food samples was weighed out into 225mls of sterile peptone water in a conical flask or beaker. The samples were washed in the saline and different quantities of the sample were added to the various volumes of both in each tube containing inverted Durham tubes. All the tube were incubated at 37^0C for 24-48 hours. After the incubation the tubes were checked for growth, acid and gas production. The estimation of the probable number of faecal coliform bacteria was done using standard and probability tables for 50ml,10ml and 1ml per 100ml of the samples (Cheesbrough, 1993). The positive tubes for coliforms by MPN technique were inoculated into *E. coli* (EC) broth and incubated at 44^0C to confirm faecal coliform (*E. coli*).

Isolation and enumeration of coliforms from emb agar: Coliforms were isolated and enumerated using the method described by APHA (1998). The microbial load was estimated by calculating the number of colonies per dilution in colony forming units/gram or milliliter.

$$Cfu/g \; or \; Cfu/ml = \frac{No.of \; colonies \times Dilution \;\; factor}{Inoculum \; volume}$$

The positive tubes from MPN technique for coliforms were inoculated into *Esherichia coli* (EC) broth and incubated at 44^oC to confirm faecal coliform (*E. coli*).

Identification of coliforms of human faecal origin (growth at 44^oC): A loopful of the isolates was inoculated into sterile MacConkey broth in tubes containing inverted Durham tubes. A change

in colour from purple to yellow indicate acid production while the presence of gaseous or air bubbles in Durham tubes show gas production.

Characterization of isolates: After isolation and purification of isolates, the growth on MacConkey agar was observed for colony morphology-size, shape, texture, inorganic colour and elevation. Gram stain was performed on the isolates using modified Newcomb's method of 1964 (Henry *et al.,* 1987). The gram-positive isolates were discarded while the gram-negative isolates were subjected to biochemical reactions according to standard methods such as sugar (Harrigan and McCance, 1976). These tests include Indole tests, Methyl red test Voges-Proskaeur test; citrate utilization test; Oxidase test, urea hydrolysis, sugar fermentation and hydrogen sulphide production.

Statistical analysis: The gram-negative, oxidase-negative isolates were subjected to statistical analysis using the analysis of variance (ANOVA).

Results and Discussion

Out of the 76 samples (66 food samples and 10 water samples), results in Table 1 showed heavy contamination of the foods with a few exceptions. There was significant difference ($p>0.05$) in the level of the individual food types using the total coliform count. Similar analyses done using the *Escherichia coli* showed a disappearance of the mentioned significance ($p<0.05$) seen in the data from the coliform count. The geometric mean count (GMC) in the foods ranged from $1.0x10^6$-$1.22x10^8$cfu/g while the GMC for coliforms (including the *Escherichia coli*) using the lactose fermenting bacteria colony count was from $1.0x\ 10^7$-$3.50x10^9$cfu/g. For the MPN technique, the GMC was from 1-180 faecal coliform per 100ml (Table 2). The high levels of the counts could be due to inadequate handling and storage of the foods by the vendors. When compared with other researchers, the values obtained were higher than the required standard for food and water. Sadiq and Abdullahi (2008) obtained high mean count of total aerobic mesophilic organism above 10^8 cfu/g from two eateries in University campus, Samaru, Zaria, Nigeria. The coliform index of the cooked foods from the eateries in Zaria showed that cooked beans had the least index ($1.0\ x10^2 – 1.3\ x\ 10^2$cfu/g) followed by rice ($1.0\ x10^2$- $1.1\ x10^3$ cfu/g).Thus, the coliform counts for the food and water in these eateries showed high coliform counts above the International Commission on Microbiological Specification of Foods (ICMSF) standards. A similar study on satchet water marketed in University of Nigeria, Nsukka campus showed that the coliform counts was higher than the US Environmental Agency Standard (Maximum contaminant levels or MCLS) for coliform bacteria which states that maximum count in any positive test should be 10 coliforms per 100ml (Dibua *et al*., 2007). These researchers obtained a mean count of $1.2\ x\ 10^{10}$ - $2.2\ x\ 10^{10}$cfu/g in cooked rice and the highest mean count of 3.3 x 1010cfu/g from both eateries in the University campus, Samaru, Zaria. These were higher than the standard stipulated by regulatory bodies. The standard set by International Commission on Microbiological Specification of Food (ICMSF) showed a limit of 10^6 cfu/g or aerobic count of foods and coliform count of less than 10^2 cfu/g (ICMSF, 1978).

The mean of the counts obtained from the assay methods (most probable number technique, *Escherichia coli* count and lactose fermenting colony count) showed that there was no significant difference ($p>0.05$) between the results obtained by the three methods. However, using the individual food types, there was significant difference ($p>0.05$) between the counts obtained from three methods respectively.

Table 1: Geometric count of lactose fermenting colonies and *Escherichia coli* in food and water samples

Sample	Food type	Water source	Storage vat	MPN No.	Lactose fermenting colonies	Lactose fermenting colonies (Ave)	*E. coli* colonies (cfu/g or ml)	*E. coli* count (cfu/ml) org.	Std. Dev. Fermenting count	Std. Dev. *E. Coli* count
B_1	Beans (cooked and plain)	Tap	Plastic pan	180	60	$6.0x10^8$	-	-	$9.89x10^7$	-
B_2	Beans (cooked and plain)	Tap	Pan	180	27	$2.7x10^8$	-	-	$8.49x10^7$	-
B_3	Beans (cooked and stewed)	Tap	Pot	0	-	-	-	-	-	-
B_4	Beans (*cooked porridge)	Borehole	Plot	0	-	-	-	-	-	-
B_5	Beans (cooked eaten with stew)	Borehole	Basin	180	62	$5.6x10^{8=}$	21	$21x10^7$	$8.49x10^7$	$5.66x10^6$
B_6	Beans (cooked and plain)	Tank	Pot	0	-		-	-	-	-
B_7	Beans (cooked and plain	Tank	Pot	1	1	$1.0x10^7$	-	-	0	-
B_8	Beans (cooked as porridge)	Tap	Pot	7	-	-	-	-	-	-
B_9	Beans (cooked on porridge)	Pump	Plate (served)	180	22	$2.2x10^8$	5	$5.0x10^6$	$2.83x10^7$	$1.41x10^6$
B_W	Water for beans	Tap/pump	Plastic-barrel	180	20	$2.2x10^8$	4	$4.0x10^2$	$1.13x10^4$	6

Y_1	Yam (cooked as porridge)	Tank	Basin	180	32	$3.2x10^8$	-	-	$6.36x10^7$	-
Y_2	Yam (cooked as porridge)	Tank	Basin	180	21	$2.x10^8$	7	$8.0 x10^6$	$4.24 x10^7$	$1.41x10^6$
Y_3	Yam (boiled and plain)	Tank	Basin	180	23	$2.3x10^8$	7	$1.1 x10^7$	$5.66 x10^7$	-
Y_4	Yam (fried)	Tank	Open basket	180	47	$4.7 x10^8$	-	-	$5.66 x10^7$	-
Y_5	Yam (with vegetable)	Borehole	Pot	0	-	-	-	-	-	-
Y_6	Yam (fried)	Borehole	Show case	0	-	-	-	-	-	-
Y_7	Yam (boiled)	Borehole	Pot	0	-	-	-	-	-	-
Y_8	Yam (boiled)	Pump	Pot	180	30	$3.0 x10^5$	6	$6.0x10^6$	$1.41 x10^7$	$1.41 x10^6$
Y_9	Yam (boiled)	Pump	Plate (served)	180	51	$5.1 x10^8$	51	$5.1 x10^7$	$8.49 x10^7$	$4.24 x10^6$
Y_W	Water for yam	Pump	Plastic-barrel	180	24	$2.4 x10^5$	48	$4.8 x10^4$	$2.89 x10^3$	$1.13 x10^4$
A_1	Abacha (mixed)	Borehole	Basin	180	300	$3.0 x10^9$	70	$7.5 x10^7$	$2.89 x10^8$	$5.66 x10^7$
A_2	Abacha (mixed)	Borehole	Basin	180	330	$3.0 x10^9$	75	$7.5 x10^7$	$2.89x10^8$	$5.66x10^7$
A_3	Abacha (plain)	Tank	Water proof	180	79	$7.9 x10^8$	-	-	$1.55 x10^8$	-
A_4	Abacha (plain)	Borehole	Water proof	90	31-	$3.1 x10^9$	225	$2.25 x10^8$	$2.26 x10^8$	$4.69 x10^7$
A_5	Abacha (plain)	Tap	Water proof	180	74	$7.4 x10^8$	11	$1.1 x10^7$	-	$4.69 x10^7$
A_6	Abacha (plain)	Tap	Water proof	180	73	$7.3 x10^8$	62	$6.2 x10^7$	$4.24 x10^7$	$1.27 x10^7$
A_W	Water for abacha	Tap	Jerry can	160	22	$2.2 x10^8$	64	$6.1 x10^8$	$4.9x10^3$	$4.2 x10^3$
O_1	Okpa (cooked)	Pump	Tin	90	350	$3.5 x10^9$	30	$3.0 x10^7$	$4.2 x10^7$	$4.44 x10^7$
O_2	Okpa (cooked)	Tank	Tin	180	-	-	-	-	$9.89 x10^7$	-
O_3	Okpa (cooked)	Tap	Water proof	0	5	-	-	-	-	-
O_4	Okpa (cooked)	Rain	Leaves	160	80	$1.5 x10^8$	150	$1.5 x10^8$	$4.2 x10^7$	$1.69 x10^7$
O_5	Okpa (cooked)	Tap	Polythene bag	0	80	-	-	-	-	-
O_6	Okpa (cooked)	Tap	Tin	180	-	$1.0 x10^6$	1	$1.0 x10^6$	0	0
O_w	Water for okpa	Tap	Jerry can	180	33	$4.3 x10^4$	43	$4.3 x10^4$	$5.66 x10^3$	$2.82 x10^3$
M_1	Moi-moi (cooked)	Pump	Can	180	7	$6.0 x10^6$	6	$6.0 x10^6$	$8.0 x10^7$	$1.0 x10^6$
M_2	Moi-moi (cooked)	Tap	Tin	0	-	-	-	-	-	-
M_3	Moi-moi (cooked)	Tank	Can	180	146	$1.1 x10^7$	11	$1.1 x10^7$	2	$2.89 x10^6$
M_4	Moi-moi (cooked)	Tap	Tine	180	-	$7.0 x10^6$	7	$7.0 x10^6$	0	$1.4 x10^6$
M_5	Moi-moi (cooked)	Tank	Tin	0	8	-	-	-	-	-
M_6	Moi-moi (cooked)	Tank	Jerry can	180	1	$1.31 x10^8$	131	$1.31 x10^8$	$1.27 x10^8$	$8.49 x10^6$
M_W	Water for Moi-moi	Tank	Water proof	0	7	-	-	-	-	-
P_1	Pears (steamed)	Tap	Water-proof	180	-	$4.0 x10^6$	4	$4.0 x10^{6-}$	0^6	$1.41 x10^6$
P_2	Pears (steamed)	Tap	Water-proof	180	28	$1.0 x10^6$	1	$1.0 x10^6$	0	$1.41 x10^6$
P_3	Pears(steamed)	Tap	Water proof	180	33	$7.0 x10^8$	7	$7.0 x10^8$	$1.41 x10^8$	$4.24 x10^6$
P_4	Pears (steamed)	Pump	Waterproof	0	10	-	-	-	-	-
P_5	Pears(steamed)	Tank	Open ray	160	-	$4.2 x10^7$	42	$4.2 x10^7$	$3.54 x10^7$	$2.83 x10^6$
P_6	Pears (steamed)	Tank	Polythene	180	11	$5.1 x10^7$	51	$5.1 x10^7$	$3.543x10^7$	$4.2 x10^6$
P_w	Pears (steamed)	Tank	Tank	90	15	$1.5x10^8$	19	-	-	-
Cf_1	Cassava foo-foo (cooked)	Tap	Pot	90	-	$1.0x10^5$	11	$1.1x10^4$	$4.03x10^4$	$1.41x10^3$
Cf_2	Cassava foo-foo (cooked)	Tap	Plate	0	11	-	-	-	-	-
Cf_3	Cassava foo-foo (cooked)	Tank	Plate	30	15	$1.1 x10^8$	-	-	$2.83 x10^7$	-
Cf_4	Cassava foo-foo (cooked)	Pump	Pan	180	-	$1.4 x10^8$	19	-	-	-

Cf_5	Cassava foo-foo (cooked)	Rain water	Pot	0	-	-	-	-	-	-
Cf_6	Cassava foo-foo (cooked)	Rain water	Plate (served)	0	-	-	-	-	-	-
Cf_W	Cassava foo-foo (cooked)	Rain	Tank	3	13	-	-	-	-	-
R_1	Rice (cooked and plain)	Tap	Plastic pan	180	-	1.3×10^5	13	1.3×10^8	1.41×10^3	4.24×10^3
R_2	Rice (jollof and stewed)	Tank	Plastic pan	0	-	-	-	-	-	-
R_3	Rice (cooked and stewed)	Tap	Plastic pan	0	51	-	-	-	-	-
R_4	Rice(cooked and jollof)	Tap	Plastic pan	180	210	5.1×10^8	4	4.0×10^6	1.13×10^8	7.07×10^3
R_5	Rice (cooked and plain	Tap	Plate (served)	160	74	-	2	-	-	-
R_6	Rice (cooked and	Tap/rain	Tank	180	24	2.1×10^6	15	2.0×10^6	3.82×10^8	2.83×10^6
R_w	Water for rice	Tap/rain	Leaves	180	53	7.4×10^8	20	1.5×10^7	1.13×10^8	3.54×10^8
Ag_1	Agidi (cooked)	Tank	Leaves	180	37	2.4×10^5	28	2.0×10^4	4.23×10^3	8.49×10^6
Ag_2	Agidi (cooked)	Tank	Leaves	180	45	5.3×10^8	30	2.8×10^7	8.49×10^9	9.89×10^6
Ag_3	Agidi (cooked)	Tank	Leaves	180	2	3.7×10^8	27	3.0×10^7	8.48×10^7	6.36×10^6
Ag_4	Agidi (cooked)	Tank	Leaves	0	19	4.5×10^8	1	2.7×10^7	7.0×10^7	1.41×10^6
Ag_5	Agidi (cooked)	Tank	Tank	0	125	2.0×10^7	-	1.0×10^6	2.12×10^7	-
Ag_6	Agidi (cooked)	Tank	Tank	180	104	1.9×10^7	64	6.4×10^7	7.07×10^8	9.89×10^6
Ag_w	Water for agidi	Tank	Bowl	180	172	1.25×10^9	88	8.8×10^4	8.89×10^3	1.84×10^3
G_1	Garri (cooked)	Tap	Plastic	180	11	9.7×10^5	122	1.22×10^8	5.66×10^7	3.54×10^6
G_2	Garri (cooked)	Tank	Pan	180	1	1.72×10^9	1	1.0×10^6	1.41×10^7	0
G_3	Garri (cooked)	Tap	Pan	0	81	1.1×10^8	1	1.0×10^6	0	0
G_4	Garri (cooked)	Tank	Pot	180	-	8.1×10^8	81	8.1×10^7	1.13×10^8	1.54×10^7
G_5	Garri (cooked)	Tap/tank	Plate (served)	0	-	-	-	-	-	-
G_6	Garri (cooked)	Tap/tank	Tank	0	-	-	-	-	-	-
G_w	Water for garri	Tap//tank	Tank	2	-	-	-	-	-	-

Key [-] ⟹ **No growth**

Table 2: Estimation of faecal coliform bacteria using the most probable number technique

Sample	Food type	Water source	Storage vat	Probability Table For The Estimation of MPN of Faecal Coliform Bacteria			
				50ml (1)	10ml (5)	1ml (5)	MPN (per 100ml of samples)
B_1	Beans (cooked and pain)	Tap	Plastic pan	1	5	5	180
B_2	Beans (cooked and plain)	Tap	Pan	1	5	5	180
B_3	Beans (cooked and stewed)	Tap	Pot	0	0	0	0
B_4	Beans (cooked and porridge)	Borehole	Plate	0	0	0	0
B_5	Beans (cooked and stewed)	Borehole	Basin	1	5	5	180
B_6	Beans (cooked and plain)	Tank	Pot	0	0	0	0
B_7	Beans (cooked and plain)	Tank	Pot	1	0	0	1
B_8	Beans (cooked and porridge)	Tap (pump)	Pot	1	2	1	7
B_9	Beans (cooked and porridge)	Tap (pump	Plate (served)	1	3	5	180
B_w	Water for beans	Tap (pump)	Plastic-barrel	1	5	5	180
Y_1	Yam (cooked as porridge)	Tank	Basin	1	5	5	180
Y_2	Yam (cooked as porridge)	Tank	Basin	1	5	5	180
Y_3	Yam (boiled/plain	Tank	Basin	1	5	5	180
Y_4	Yam (fried)	Tank	Open basket	1	5	5	180
Y_5	Yam (with vegetable)	Borehole	Pot	0	0	0	0
Y_6	Yam (boiled)	Borehole	Show case	0	0	0	0
Y_7	Yam (boiled)	Borehole	Pot	0	0	0	0
Y_8	Yam (boiled)	Pump	Pot	1	5	5	180
Y_9	Yam (boiled)	Pump	Plate (served)	1	5	5	180
Y_w	Water for yam	Pump	Plastic-barrel	1	5	5	180
A_1	Abacha (mixed)	Borehole	Basin	1	5	5	180

A_2	Abacha (mixed)	Borehole	Basin	1	5	5	180
A_3	Abacha (plain)	Tank	Water-proof	1	5	5	180
A_4	Abacha (plain)	Borehole	Water-proof	1	5	3	180
A_5	Abacha (plain)	Tap	Water-proof	1	5	5	180
A_6	Abacha (plain)	Tap	Water-proof	1	5	5	180
A_w	Water for abacha	Tap	Jerry can	1	5	4	160
O_1	Okpa (cooked)	Pump	Tin	1	5	3	90
O_2	Okpa (cooked)	Tap	Tin	1	5	5	180
O_3	Okpa (cooked)	Tap	Water-proof	1	0	0	0
O_4	Okpa (cooked)	Tap	Leaves	1	5	4	160
O_5	Okpa (cooked)	Pump	Polythene bag	0	0	0	0
O_6	Okpa (cooked)	Tank	Tin	1	3	3	18
O_w	Water for okpa	Tap	Jerry can	1	5	5	180
M_1	Moi-moi (cooked)	Rain	Can	1	5	5	180
M_2	Moi-moi (cooked)	Tap	Tin	0	0	0	0
M_3	Moi-moi (cooked)	Tap	Can	1	5	5	180
M_4	Moi-moi (cooked)	Tap	Can	1	5	5	180
M_5	Moi-moi (cooked)	Pump	Tin	0	0	0	0
M_6	Moi-moi (cooked)	Tap	Tin	1	5	5	180
M_W	Water for moi-moi	Tank	Jerry can	0	0	0	0
P_1	Pears (steamed)	Tap	Water proof	1	5	5	180
P_2	Pears(steamed)	Tap	Water proof	1	5	5	180
P_3	Pears(steamed)	Tap	Water-proof	0	0	0	0
P_4	Pears(teamed)	Pump	Water-proof	1	5	5	180
P_5	Pears(steamed)	Tank	Open tray	1	5	4	160
P_6	Pears(steamed)	Tank	Waterproof	1	5	5	180
P_w	Water for Pears	Tank	Tank	1	5	3	90
Cf_1	Cassava foo-foo (cooked)	Tap	Pot	0	0	0	0
Cf_2	Cassava foo-foo (cooked)	Tap	Plate	1	4	3	30
Cf_3	Cassava foo-foo (cooked)	Pump	Plate	1	4	3	30
Cf_4	Cassava foo-foo (cooked)	Tank	Pan	0	0	0	0
Cf_5	Cassava foo-foo (cooked)	Tank	Pot	0	0	0	0
Cf_6	Cassava foo-foo (cooked)	Tank	Plate (served)	0	3	0	0
Cf_W	Water for cassava foo-foo	Tap	Tank	1	5	5	180
R_1	Rice (cooked and plain)	Tank	Plastic pan	0	0	0	0
R_2	Rice (jollof and stewed)	Pump	Plastic pan	0	0	0	0
R_3	Rice (cooked and stewed)	Rain water	Plastic pan	1	5	5	180
R_4	Rice (cooked and jollof)	Rain water	Plastic pan	1	5	4	160
R_5	Rice (Cooked and plain)	Rain	-	1	5	5	180
R_6	Water for rice	Tap	Plate (served)	1	5	5	180
R_W	Agidi (cooked)	Tank	Tank	1	5	5	180
Ag_1	Agidi (cooked)	Tap	Leaves	1	5	5	180
Ag_2	Agidi (cooked)	Tap	Leaves	1	5	5	180
Ag_3	Agidi (cooked)	Tap/rain	Leaves	0	0	0	180
Ag_4	Agidi (cooked)	Tap/rain	Leaves	0	0	0	0
Ag_5	Agidi (cooked)	Tank	Leaves	1	-	5	0
Ag_6	Agidi (cooked)	Tank	Tank	1	5	5	180
Ag_w	Water for agidi	Tank	Tank	1	5	5	180
G_1	Garri (cooked)	Tap	Bowl	1	5	5	180
G_2	Garri (cooked)	Tank	Plastic	1	5	5	180
G_3	Garri (cooked)	Tap	Pan	0	0	0	0
G_4	Garri (cooked)	Tank	Pan	1	5	5	180
G_5	Garri (cooked)	Tap/tank	Pot	0	0	0	0
G_6	Garri (cooked)	Tap/tank	Plate (served)	0	0	0	0
G_w	Water for garri	Tap/tank	Tank	0	1	1	2

Note: 50mls and 10mls ⟶ volumes of the broth in the tubes ( ) ⟶ number of the tubes

O ⟹ No growth

This work reported here is a part of a wider study on the bacteriological quality of food and water in Nsukka area of Enugu State of Nigeria. The foods consumed in the open-air market, restaurants and cafeterias around the town were studied in order to determine the level of hygiene, sanitation and manufacturing practice. The food items of interest were those locally prepared and eaten by persons outside their homes. The high counts recorded for almost all the food items could be attributed to the fact that the cooked foods are not usually maintained at ambient temperature for a long time and the content coupled with general poor storage facilities. Byran(1988) had stated that general state of inadequate hygiene and sanitation could count for high counts of microorganisms in foods. Abdullahi *et al. (*2004) andSadiq and Abdullahi (2008) all reported the presence of large microbial population in some foods due to bad hygienic practices by food handlers. They were generally high aerobic count of the food handlers and all the food items, which were an indication of poor hygienic state of the food service centres.

Coliform bacteria were used in this study as indicators of faecal contamination and possible presence of pathogenic organisms in the food groups and a few water samples. The water samples were used specifically to trace the sources of contamination of the foods. The two methods were used to enumerate the number of coliforms present in the food and water samples. The use of these three methods ensured more efficient recovery of the coliforms. Besides use of MPN and direct plate methods the validity of the result would be strengthened and the problem of false negative in food and water samples would greatly be reduced. The results got by MPN technique were not significantly different ($p<0.05$) from those got by serial dilution (duplicate plate) method.

LeChavellier and McFeters (1984) had pointed out that data from only one method is not significant for proper analysis of food and water samples. Though membrane filtration is the ideal method for estimating the number of coliforms in food and water because of its accuracy and speed (Cheesbrough, 1993), It was not used in this work because of the high cost and unavailability of the membrane. Windle (1968) noted that a three-hour resuscitation at 37^0C prior to incubation at 44^0C narrowed the gap in true positive obtained between plate count dilution techniques and membrane filtration. It is suggested that a combination of MPN and plate count dilution techniques with resuscitation might achieve up to 99.0% correlation.

From the MPN techniques, the ideal situation would be one in which the water contains no coliforms. However, according to the World Health Organization (1971), 10 coliforms per 100ml of water is the upper limit of contamination acceptable in drinking water in small community supplies. In our observation, the coliform counts in the water samples were greater than the recommended world standard. This implies that most of the water supplies in Nsukka do not meet the acceptable hygienic standard. With the advent of 'pure water' (packaged water), many consumers had resorted to the use of sachet or bottled water during food consumption .However, a study within Nsukka metropolis (the University) indicated that the sachet water did not conform to regulatory standards because the pathogens isolated (coliforms) were very high (Dibua *et al*., 2007).

The research emphasized on the need to increase awareness of the hazard on eating contaminated foods and ways to prevent the contamination. Thus, microbiological quality control of foods and water is essential for a safe, wholesome and consistent food supply. This could be achieved with the aid of the governmental public health worker in cooperation with the food sellers. Routine inspection of premises where foods are cooked or sold and samples of the food for microbiological examination should be encouraged (Ahmed *et al.,* 1976). The involvement and active participation of individuals and communities will help achieve this. There was high coliform index for the different food items and water that were served in the cafeteria and its environs on campus. This of course should be of great concern bearing in mind that WHO (1981) had recommended that drinking water should have a coliform index less than one per 100ml of treated water. The high coliform index may be due to leakages in the water supply network and other pollution that bring about an increase in the microbial population (Zammelli *et al.,* 1993). It could as well be attributed to the shortage pattern of water and the available storage devices.

Indeed, since the coliform bacteria are used to indicate the presence of all pathogenic organisms transmitted by faecal-oral route, they should be done routinely in bacteriological laboratory according to LeChavellier and McFeters (1984). The more frequent monitoring will be helpful in detecting contaminated water and food supplies. Here in Nigeria, there is no generally established microbiological standard for vended food and water. In the United States of America, the U.S. department of the Interior, Federal Water Pollution Control Administration in its 1968 report on water quality stated the tolerable limit for recreation purposes as up to ten as the total faecal coliform in 100ml of water (Black *et al.,* 1980). Adoption of this standard would be beneficial in this country. The isolation of *E.coli* gives an indication of a faecal pollution with an attendant risk of other pathogens like Salmonella species and other parasites that may be transmitted through water or food. Zweitering (2002) had reported that pathogens have been transmitted through water. Adesiyun (1995) had earlier reported that water for consumption and other drinks are highly contaminated with coliforms in some parts of Trinidad. With the high coliform index, there is the potential of spreading food borne diseases.

In conclusion, a change in the coliform monitoring technique for food and water supplied to the public and restaurants will be helpful. The improvement on the methods for coliform determination will be an effective rationale to

achieve a better control of pollution and diseases outbreak. Also, the results indicated that people who patronize the food vendors are obviously exposing themselves to hazards. It is imperative that good sanitation be maintained in food preparation and handling of cooked foods at all times to save consumers from the possible dangers of food-borne illnesses.

References

Abdullahi, I.O., Umoh, V.J, Ameh, J.B. and Galadima ,M. (2004). Hazards associated with kilishi preparation in Zaria, Nigeria. *Nigerian Journal of Microbiology*, **18**(1/2)**:** 339-345.

Adam, M.R and Moss, M.O. (1996). Methods for the Microbiological Examination of Foods. In: Food Microbiology, Royal Society of Chemistry, Cambridge, pp 303-322.

Adesiyun, A.A.(1984). Prevalence and characterization of Staphylococcus from five ready-to-eat products in Nigeria. *Nigerian Food Journal,* **2**(2): 135-139.

Adesiyun, A.A. (1995). Bacteriological quality of some Trinidadian ready-to-consume foods and drinks and possible health risks to consumers. *Journal of Food Protection*, **58**: 651-655.

Adesiyun, A.A. and Kwaya, J.K.P. (1983). Microbial quality and potential of Staphylococcal growth in some consumed constituted infant food. *Nigerian Food Journal,* **1**(1): 1118-120.

Ahmed, Z.I., Poahmi, A. and Siddiqui, S.C. (1976). Bacteriology examination drinking water of Karachi and Isolation of enteric pathogens. *Pakistan Journal of Science Industrial Research,* **7**:103-110.

Altekruse, S.F., Street, D.A., Flein, S.B. and Levy, (1995). Consumer knowledge of food borne microbial hazards and food handling practices. *Journal of Food Protection,* **5**: 66-73.

APHA (1998). Analytical Methods for Total Coliforms. In: Standard Methods for the Examination of Water and Wastewater, American Public Health Association.

Black, RA.S..E., Merson, M.H., Rahman, A.S.M., Yunus, M., Alim, A.R., Uhg, M.I., Holken, I. and Culin, R.H. (1980). Viral and parasitic agents associated with diarrhoea in rural, Bangladeshi *Journal Infectious Diseases,* **142:** 660-664.

Brooks, G.F., Butel, J.S. and Morse, S.A. (2004). Enteric Gram Negative Rods (Enterobacteriaceae). In: Jawetz, Melnick and Adelberg's Medical Microbiology. 23rd Int'l Edition. McGraw Hill Education Companies Inc. Singapore, pp 248-261.

Byran, F.L.C. (1988). Risks of practices, procedures and processes that lead to outbreaks of food borne diseases. *Journal of Food Protection,* **59**:287-294.

Cheesbrough, M. (1993). Bacteriological Testing of Water Supplies: Probability Tables for Estimating with MPN of Faecal Coliform Bacteria. Medical Laboratory Manual for Tropical Countries. Educational Low Priced Books Scheme (ELBS). pp 219-220.

Clark, J.A. and Pagel, J.C. (1997). Pollution indicator bacteria associated with municipal raw water and drinking water supplies. *Canadian Journal Microbiology,* **23:**465-470.

Davidson, S., Passmore, R., Brook, J.F. and Truswell, A.S. (1975). Human Nutrition and Dietetics. 6th Ed., Churchill Livingstone, Edinburgh.

Dibua, U.E., Esimone, C.O. and Ndianefo, P.C. (2007). Microbiological and physiochemical characterization of satchet water samples marketed in Nsukka Campus of the University of Nigeria. *Bio-Research,* **5**(1): 189-193.

Dosumu, O.O., Oluwanyi, O.O., Awolola, G.V. and Okunola, M.O. (2009). Stability studies and mineral concentrations of some Nigerian packed fruit juices, concentrate and local beverages. *African Journal of Food Science,* **3**(3): 082-085.

Enwere, N.J. (1998). What is Food? In: Foods of Plant Origin, Afro-Orbis Publ. Ltd., Nigeria, pp 3-21.

Feng, P.C.S. and Hartman, P.A.C. (1982). Fluorogenic assays for immediate confirmation of *Escherichia coli. Applied and Environmental Microbiology,* **43**(6): 1320-1329.

Frazier, W.C. and Westhoff, D.C. (2005). Food as a Substrate for Microorganisms. In: Food Microbiology. 4th Ed., Tata McGraw-Hill Publ. Co. Ltd., New Delhi. pp 3-16.

Harrigan, W.F. and McCance, M.E. (1976). Biochemical tests for Bacteria. In: Laboratory Methods in Food and Dairy Microbiology , Academic Press, London. pp 66-82.

Henry, W., Sealey, J. and Demark, P.J.V. (1987). Staining Techniques: Gram Stain. In: Selected Exercises from Microbes in Action, 2nd Ed. W.H. Freeman and Co., USA.

ICMSF (1978). International Commission on Microbiological Specification of Foods 2: Sampling for Microbiological Analysis Principles and Specific applications. University of Toronto Press. pp 119-118.

Jay, J.M. (2004). History of Microorganisms in Food. In: Modern Food Microbiology. 4th Ed. CBS Publ., New Dehli. pp 3-10.

Jiwa, S.F., Krovacek, K. and Wadstrim, T. (1981). Enterotoxigenic bacteria in food and water from an Ethiopian Community. *Applied and Environmental Microbiology.* **41**(4): 3-16.

LeChavellier, M.W. and McFeters, G.A. (1984). Recent advances in coliform methodology for water analysis. *Journal of Environmental Health,* **47**(1): 5-9.

Mahale, D.P., Khade, R.G. and Vaidya, V.K. (2008). Microbiological analysis of street vended fruit Juices from Mumbai City, India, *Internet Journal of Food Safety*, **10**:31-34.

Mermin, J.H., Villar, R., Carpenter, J., Robert, L., Samaridden, A., Gasanova, L., Lomakina, S., Bopp, C., Hutwagner, L., Mead, P., Ross, B. and Mintz, E.D. (1999). A massive epidermic of multi-drug-resistant typhoid fever in Tajikistan Association with consumption of municipal water. *The Journal of Infectious Diseases,* **176**: 1416-1422.

Okaka, J.C. and Ene, G.I. (2005). Microorganisms–Implications of Presence in Foods. In: Food Microbiology- Methods in Food Safety Control, Ocjanco Academic Publication, Nigeria. pp 1-12.

Oxoid (1982). The Oxoid Manual of Culture Media, Ingredients and Other laboratory Services, 5th Ed., Oxoid Limited, Basingstoke.

Prescott, L.M., Harley, J.P and Klein, D.E. (1996). The Study of Microbial, Structure, Microscopy and Specimen Preparation. In: Microbiology, 7th ed. McGraw-Hill, New York. pp 17-36.

Sadiq, M. and Abdullahi, I.O. (2008). Hygienic evaluation of two food service centres in a University campus in Samaru, Zaria, Nigeria. *Nigerian Food Journal,* **26**(1): 71-76.

Speck, M.L. (Ed.) (1988). Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association ,Washington D.C.. pp 200-500.

WHO (1971). International standard of drinking water. *WHO Journal,* pp 3.

WHO(1981). Global Strategy for Health for all by the year 2000. WHO, Geneva, pp7-13.

WHO(1993). Contaminated Food: A Major Cause of Diarrhoea associated with Malnutrition among Infants and Young Children. *WHO Journal*

Windle, T.E. (1968). The Examination of Water and Water Supplies. 42nd Report of the Metropolitan Water Boards, 7th Ed; Churchill Publ., London, pp 117.

Zammelli, P.Z., Brabbia, C.A. and Gathia, G.E. (1993). Air pollution associated with food spoilage. *Journal of Food Protection,* **12:**105-109.

Zweitering,M.H.(2002). Quantification of Microbial quality and safety in minimally processed food. *Dairy Journal,* **12**:264-271.