

The Bacteriology of Fried Ready-To-Eat Foods Sold In Enugu Metropolis, Nigeria

*Ochei Kingsley Chinedum¹, Obeagu Emmanuel Ifeanyi², Aniukwu Vivian Ebere³ and Mbajiuka Chinedu Stanley⁴

1. Department of Medical Laboratory Sciences, Faculty of Basic Medicine, Ambrose Ali University Ekpoma, Edo State, Nigeria.

2. Diagnostic Laboratory Unit, University Health Services Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

3. Department of Medical Laboratory Sciences, College of Medicine, University of Nigeria, Enugu Campus.

4. Department of Microbiology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Abstract: A research to investigate the bacteriology of four selected fried ready-to-eat food types (Yam, Potato, Akara and Plantain) sold freely and openly in various zones in Enugu metropolis between May and September, 2011 was carried out. Of the 160 samples examined, 151(94.4%) were contaminated by various bacterial agents. Of the 151 contaminated, 91(60.3%) were sampled in the morning, while 60(39.7%) were sampled in the evening, and the difference between the total number of isolates in the 2 periods was statistically significant ($P < 0.05$). Of the 151 positive samples, 47(31.1%) were bought from the covered source (show cases) while 104 (68.9%) were purchased from uncovered source, which difference was also statistically significant ($P < 0.05$). Of the 151 positive samples, Diphtheroids contaminated the highest number of samples with 41(27.2%), followed by *Bacillus subtilis* 31(21.0%), *Streptococcus fecalis* and *Klebsiella aerogenes* were the least with 2(1.3%) each. Of the 151 positive samples, 135(89.4%) were contaminated by Gram positive organisms while 16(10.6%) were Gram negative, and the difference was also statistically significant ($P < 0.05$). Mean bacterial count of the bacterial isolates showed that fried yams had the highest count of 2.8×10^2 with a contamination rate of 40(100%) while fried plantain had the lowest mean count of 1.0×10^2 with a contamination rate of 40(100%), statistically, there was significant difference in the bacterial counts of the different food types ($P < 0.05$). Generally, a total of 7 bacterial genera were encountered, and there was statistically no significant difference in generic distribution according to food types ($P > 0.05$). These findings suggest that the consumption of commercially prepared fried ready-to-eat foods sold in Enugu may have some public health implications. A controlled study involving more types of ready-to-eat foods from more areas over a longer period of time is hereby advocated in order to institute a regulatory measure. Standardization regimen is also recommended for the welfare of ever increasing consumers.

Keywords: Bacteriology, Fried foods, gram positive, gram negative and contamination rate.

I. Introduction

Food is any substance usually composed of carbohydrate, fats, proteins and water that can be eaten and/or drunk by an animal or human for nutrition or pleasure [1]. Ready-to-eat food is the food that is ready for immediate consumption at the points of sale [2].

Food we purchase are not sterile in the sense that they normally contain germs (bacteria, viruses, yeast and moulds), some of which can lead to food intoxication and infections when present above the acceptable levels [3]. Also foods harbor a variety of micro organisms, bacteria and fungi which are ubiquitous and are especially plentiful in soil and around us (air) and could easily contaminate foods [4]. This could also result from improper protection of foods from flies, which may carry food borne pathogens.

However, contamination and growth of pathogens such as *staphylococcus aureus*, *salmonella* species, *Bacillus* species, *pseudomonas aeruginosa*, *Clostridium* species, *vibrio cholerae* and *Escherichia coli* can result in perceptible changes in quality of food. Some foods can transmit a wide range of diseases in a condition termed food infection, where the food serves as a vehicle for the transfer of the pathogen to the consumer, in whom the pathogen grow and causes disease [5]. Another condition that might arise in food intoxication, where the pathogens grow in the food and produce toxins that can affect the consumer of the food [6]. While food borne diseases remain an important public health problem worldwide, one of the most significant food safety hazards is associated with foods of animal origin [7].

A number of observational studies have shown that these foods are sometimes held at improper temperatures, excessively handled by food vendors and sold at dirty surroundings [8]. Documented evidences have continued to link pathogenic micro organisms in food to incidences of food borne diseases and

intoxications.

Food borne illness caused by microbial contamination of foods is an important international public health problem with consequent economic reduction [9], and is known to be a major cause of diarrhea diseases especially in developing countries [10].

In spite of numerous advantages offered by ready-to-eat foods, there is also several health hazards associated with this sector of the economy. Multiple lines of evidence reveal that foods exposed for sale on road sides may become contaminated either by spoilage or pathogenic micro organisms [11, 12, 13 and 14]. Evidently, street vended foods have shown epidemiological links with illness [15 and 16]. Food safety depends on their adequate manipulation, transportation and storage. Those mostly susceptible to food infections and or intoxications are children, elderly and immunosuppressed individuals [17].

In February and March 1998, Northern Nigeria faced a meningitis outbreak of unprecedented magnitude. This outbreak during which 109,580 cases were reported by Mohammed et al. (2000) overwhelmed the curative health care system and prompted a major intervention from the Nigeria ministry of health with the assistance of non-governmental organizations, including Medecins Sans Frontieres (MSF). In amidst of this outbreak the state of Kano (1996 population: 4,931,789) was struck by another large-scale outbreak of severe diarrhea that was soon confirmed to be cholera, as two stool samples examined at the French National Reference Centre for Vibrios and cholera grew *Vibrio cholera* sero type 01, serotype Inaba.

According to report by Sarah et al., between 1992 and 1999, 60 outbreaks of food borne infectious intestinal disease associated with the consumption of salad items, fruit and vegetables, were recorded in England and Wales. Two thousand one hundred and seventy people were affected and 27 were admitted to the hospital. No death was recorded. In 17 of the outbreaks more than 50 people were affected.

Food borne microbiologic hazards may be responsible for as many cases of illness as possible each year and are thus an important food safety challenge. To lower the incidence of food borne disease, many experts and stakeholders urge the development of science and risk-based food safety system in which decision makers prioritize hazards and interventions and reduction of risks [18]. Such a system requires an understanding of many risk factors between the point of production and the point of consumption and ability to systematically target intervention efforts along this “farm-to-fork” continuum [19].

However in Nigeria, a number of foods have been reported to have high incidence of bacteria [20], [21], [22], [23], [24] and [25]. But there is limited information on the health challenges from food borne diseases from ready-to-eat foods retailed within a highly populous community. It is in view of this that this study was conducted in attempts at filling this gap with the following:

AIMS AND OBJECTIVES

1. To examine and isolate micro-organisms (bacteria) from fried ready-to-eat foods like yam, plantain, beans ball and plantain obtained from different vendors within Enugu metropolis.
2. To identify such organisms by standard laboratory methods.
3. To access the level of contamination by carrying out microbial viable count.
4. To determine possible predisposing factors and then proffer valid recommendations based on the findings.

II. Materials And Methods

SAMPLE COLLECTION / DESCRIPTION

A total of 160 samples comprising of four different fried food types (Yam, potato, plantain and akara (beans ball) collected in 2 batches (morning and evening), were bacteriologically analyzed. These foods were sourced from different areas in Enugu Metropolis (Asata, Abakpa, Gariki, Obiagu, and New Heaven) in the same quantity. Fourty (40) samples of each food type were purchased in sterilized brown bag envelopes directly from sellers and immediately brought to the laboratory for analysis.

For each fried food type, samples were purchased in batches (5 pieces per batch). These foods were usually displayed immediately after frying to drain oil and cool off in trays and also to attract consumers, while some were put inside enclosed glass cupboards (show cases) after draining oil, but usually opened on top at frequent intervals for sales.

These sliced fried foods are usually sold to customers using “chop sticks” to select a preferred piece. Sellers are usually positioned in strategic junctions as bus stops where they will be patronized by the numerous customers rushing off for work in the morning including pupils and students rushing for school or returning. These positions are chosen where customers embark or disembark from buses\motorcycles.

Table 1:

Distribution Of Fried Food Types Sampled In The Study

ZONES	FOOD TYPES (IN BATCHES)				
	YAM	POTATO	AKARA	PLANTAIN	TOTAL
ASATA	8	8	8	8	32
ABAKPA	8	8	8	8	32
GARIKI	8	8	8	8	32
OBIAGU	8	8	8	8	32
NEWHAVEN	8	8	8	8	32
TOTAL	40	40	40	40	160

In all the zones, 8 batch samples each were used for a particular food type and total of 32 samples from the 5 zones each.

For all the food types, a total of 40 batch samples each was used, 8 from each zone.

All together, 160 batch samples of four different food types sold in Enugu were examined (yam, potato, akara and plantain).

ISOLATION / COUNTS OF BACTERIA

Ten (10g) of food were weighed into a sterile container, 10ml diluent (sterile peptone water) added, and shaken well for 20minutes.

Serial 10 fold dilutions of the diluent was incubated for 1hour and each sample was inoculated unto freshly prepared and well dried Blood agar, MacConkey agar (Oxoid) and Salmonella shigella (SS Difco) agar plates using a standard wire loop. (Methods were according to Mackie and McCartney, 1989; Collee, et.al, 1989). Each batch was normally pooled together to give more chances of positive isolation.

Each plate was carefully labeled using grease pencils, recording food types and place of purchase for easy processing. The agar plates were incubated aerobically at 37°C for 24 hours. Bacteria isolates were then counted after the incubation and identified using standard bacteriological methods.

IDENTIFICATION OF ORGANISMS ISOLATED

Organisms isolated from the culture media after overnight incubation were identified using the following methods:

- Plate reading
- Gram technique
- Biochemical test. (Collee et al.,1989).

PLATE READING

Plates were carefully brought out of the incubator and examined under bright light for evidence of haemolysis on blood agar plates, lactose fermentation on MacConkey agar plates, sizes of colonies, pigmentation, consistency, odour and elevation of colonies, including growth and their features on Salmonella and Shigella agar.

GRAM TECHNIQUES

The Gram staining reaction was used to help identify isolates in culture (gram positive or gram negative organisms). Using a sterile wire loop, a pure culture from a discrete colony on the culture plate was emulsified in a drop of normal saline on grease free clean microscope slides.

PROCEDURE

- The dried smear was fixed.
- The fixed smear was covered with crystal violet stain for 30 seconds.
- The stain was rapidly washed off with clean water.
- All the water was tipped off, and the smear covered with lugol's iodine for 30 seconds.
- The iodine was washed off with clean water.
- The smear was decolorized rapidly for few seconds using acetone and washed immediately with clean water.
- The smear was covered with neutral red stain for 60 seconds.
- The stain was washed off with clean water.

- The back of the slide was washed clean and placed in a draining rack for the smear to air dry.
- The smear was then examined microscopically with oil immersion objective to report the bacteria and cells.

RESULT:

Gram positive bacteria - Dark purple
Gram negative bacteria - Dark red

MOTILITY TEST

Knowing whether an organism is motile or non-motile can often assist in its identification. Here, the hanging drop method was used from a broth culture of the isolate.

Hanging Drop Preparation

1. A drop of suspension was placed on a cover glass and inverted over a normal slide supported on a ring of plasticine.
 2. The preparations were microscopically examined for motile organisms, using 10x and 40x objectives.
- Note: The movement of small motile bacteria must be distinguished from the Brownian motion which is shown by all micro-organisms and particles when suspended in a fluid. True bacteria motility is the ability of an organism to move itself in different directions or a single direction.

BIOCHEMICAL TESTS

INDOLE TEST

This test demonstrates the ability of certain bacteria to decompose the amino acid, tryptophan to indole, which accumulates in the medium.

PROCEDURE

1. The test organism was inoculated in a bijou bottle containing 3ml of sterile peptone water.
2. It was incubated at 35-37⁰c for up to 48hours.
3. 0.5ml of kovac's reagent was added to test for indole and the contents was gently shaken. It was examined for a red colour in the surface layer within 10 minutes.

RESULT:

Appearance of red colour in the alcohol layer indicated a positive reaction.

METHYL RED TEST

Some bacteria when cultured in buffered glucose peptone water produce much acid from the fermentation of glucose to give red colour with the methyl red indicator.

PROCEDURE

A small quantity of the organism was inoculated into the sterile glucose phosphate peptone water medium and incubated at 37^oc for 48 hours. Then 5 drops of the methyl red reagent was added into the culture after incubation, mixed and read immediately.

RESULT:

Positive test showed red colour while negative test showed yellow colour.

VOGES – PROSKAUER TEST

Many bacteria ferment carbohydrate especially glucose phosphate peptone medium with the production of acetyl methyl carbinol (acetoin) which can be detected by an oxidation reaction.

PROCEDURE

2ml of sterile glucose phosphate peptone was inoculated with the test organism then incubated at 37^oc for 48 hours. 1ml of 40% potassium hydroxide and 3ml of 5% solution of alphanapthat in absolute alcohol was added after incubation. The tube was shaken at interval to ensure maximum aeration.

RESULT:

A positive action showed a pink colour in 2 – 5 minutes.

CITRATE UTILIZATION TEST

This test is used occasionally to assist in the identification of enterobacteria. It is based on the ability of an organism to use citrate as its only source of carbon and ammonia as its only source of nitrogen.

PROCEDURE

Here, Simon's citrate agar medium was used. The sterile medium was inoculated from a saline suspension of the test organism and incubated for 96 hours at 37^oc.

RESULT:

A blue colour and streak of growth indicated a positive reaction while the original green colour and no growth are indicative of a negative reaction.

TEST FOR ENZYMES

CATALASE TEST

This test is used to differentiate those bacteria that produce the enzyme catalase, such as staphylococcus from non-catalase producing bacteria such as streptococcus. Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water.

PROCEDURE

2-3ml of the hydrogen peroxide solution was poured into a test tube while a small quantity of the test organism was picked and immersed into the tube containing the hydrogen peroxide.

RESULT:

The production of gas showing the release of oxygen is a positive test while negative result is when there is no bubble.

COAGULASE TEST

This test is used to differentiate pathogenic Staphylococcus aureus from non-pathogenic staphylococcal strains. Coagulase causes plasma to clot by converting fibrinogen to fibrin.

PROCEDURE

A drop of physiological saline was placed on a clean microscope slide. Then a colony of the test organism was emulsified in the drop of saline to form a smooth milky suspension. A drop of plasma was added to the suspension and was gently mixed.

RESULT:

Clumping within 10 seconds is positive test, while no clumping is negative reaction.

OXIDASE TEST

This test is used to identify organisms that are strongly oxidase positive. This test depend on the presence in bacteria of certain oxidases that will catalyze the transport to electrons between electron donors in the bacteria redox dye,teramethyl-p-phenylene diamine dihydrochloride(the reagent used)

PROCEDURE

1. A piece of filter paper was placed in a clean petridish and 2 or 3 drops of freshly prepared oxidase reagent added.
2. A piece of stick or glass rod was used to remove a colony of the test organism and smeared on the filter paper.
3. It was observed for the development of a blue-purple colour within a few seconds.

RESULT:

Development of blue-purple colour within a few seconds indicates positive test while negative is no blue purple colouration.

UREASE TEST

Testing for urease enzyme activity is important in differentiating enterobacteria based on their ability to produce urease enzyme. The test organism is cultured in a medium which contains urea and the indicator phenol red. When the strain is urease – producing, the enzyme will break down the urea by hydrolysis to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline as shown by a change in colour of the indicator to pink-red.

PROCEDURE

The medium Christensen's medium, was prepared and autoclaved at 121°C for 30 minutes, pH 6.8 glucose and urea sterilized by steaming at 100°C for 15 minutes were added and mixed, then poured into tubes as deep slopes. The test organism was inoculated on the agar slopes and incubated at 37°C for 4 hours and then over night.

RESULT:

Change of colour to purple pink is indicative of urease positive.

SUGAR FERMENTATION TEST

The medium used was peptone water at p.H. 7.2, with bromocrysol blue as an indicator. This sugar test for acid and gas production or only acid production.

PROCEDURE

Into a bijour bottle containing an inverted Durham tube for gas-collection, 3ml of the medium was added. The bijour bottles were autoclaved at 121°C for 15 minutes to remove air from the tube and then cooled to 50°C before adding any sugar. The sugars were glucose, mannitol and sucrose. Each bijou bottle was incubated for 18-24 hours at 37°C.

RESULT:

Presence of colour change indicates acid formation while gas formation in the Durham tube indicates the ability of the organisms to produce gas.

III. Results

Seven Genera (8 bacterial isolates) were recovered from 160 samples of the different fried food types examined; Diphtheroids, Bacillus, Lactobacillus, Staphylococcus, Escherichia, Streptococcus and Klebsiella.

The bacterial species were; Diphtheroids, Bacillus subtilis, Lactobacillus acidophilus, Coagulase negative Staphylococcus, Escherichia coli, Staphylococcus aureus, Bacillus cereus, Streptococcus faecalis and Klebsiella aerogenes.

Table 2:
Distribution Of Bacterial Isolates From Four Selected Fried Foods Sold In Enugu

FOOD TYPE	BACTERIAL ISOLATES
A - YAM (FRIED SLICES)	Diphtheroids (12), Lactobacillus (4), Bacillus subtilis (8), Bacillus cereus (3), Staphylococcus aureus(5), Coagulase -ve Staphylococci (4), Escherichia coli (4)
B - POTATO (FRIED SLICES)	Diphtheroids (10), Lactobacillus (10), Bacillus subtilis (4), Bacillus cereus (3), Staphylococcus aureus (2), Coagulase -ve Staphylococci (3), Escherichia coli (3),
C - AKARA (FRIED BEAN CAKE)	Diphtheroids (9), Lactobacillus (7), Bacillus subtilis (9), Bacillus cereus (3), Staphylococcus aureus (2), Coagulase -ve Staphylococci (2), Escherichia coli (2), Klebsiella (2).
D - PLANTAIN (FRIED SLICES)	Diphtheroids (10), Lactobacillus (6), Bacillus subtilis (10), Staphylococcus aureus (2) Coagulase -ve Staphylococci (5), Escherichia coli (5), Streptococcus (2).

Table 3:

Food Contamination By Bacteria Genera

BACTERIA GENERA	NO. OF FOODS CONTAMINATED	PERCENTAGE (%)
Diphtheroids	41	27.2
Bacillus	40	26.5
Lactobacillus	27	17.9
Staphylococcus	25	16.6
Escherichia	14	9.3
Streptococcus	2	1.3
Klebsiella	2	1.3
TOTAL	151	94.4%

Table 3 shows the frequency of bacteria isolates from the 160

Samples of the 4 selected ready to-eat-food types examined in Enugu. Out of the 160 samples analyzed, 151 (94.4%) yielded bacteria growth while 9 yielded no growth. Of the 151 samples that yielded growth, 78 samples yielded pure growth of one specie each, while 73 yielded mixed growth, thus a total of 7 bacteria genera were isolated. Of the 151 positive samples; 41 (27.2%) yielded Diphtheroids, 40 (26.5%) yielded Bacillus, 27 (17.9%) Lactobacillus, 25 (16.6%) Staphylococcus, 14 (9.3%) Escherichia coli, 2(1.3%) yielded Streptococcus and Klebsiella each.

Table 4:
Frequency Of Bacterial Organisms Isolated From Food Samples Examined (Mixed Growth)

BACTERIA ISOLATES	FREQUENCY	PERCENTAGE(%)
Diphtheroids	50	27.0
Lactobacillus acidophilus	30	16.2
Bacillus subtilis	26	14.1
Coagulase negative Staphylococcus	20	11.0
Bacillus cereus	18	10.0
Escherichia coli	17	9.2
Streptococcus faecalis	13	7.0
Staphylococcus aureus	9	5.0
Klebsiella aerogenes	2	1.1
TOTAL	185	100

Table 4. shows the frequency of Bacteria isolates from the fried food samples examined. Of the samples that yielded pure growth and mixed growth, a total of 185 isolates were obtained. Of the 185 isolates, Diphtheroids ranked the highest with 50 (27.0%), followed by Lactobacillus acidophilus with 30 (16.2%), Bacillus subtilis 26(14.1%), Coagulase negative Staphylococcus 20(11.0%), Bacillus cereus 18(10.0%), Escherichia coli 17(9.2%), Streptococcus faecalis 13(7.0%), Staphylococcus aureus 9(5.0%) and Klebsiella aerogenes was the least 2(1.1%).

Table 5:
Distribution Of Bacterial Genera According To Fried Food Types Examined

GENERA	YAM	POTATO	AKARA	PLANTAIN	TOTAL
Diphtheroids	12(29.3%)	10(24.4%)	9(22.0%)	10(24.4%)	41(27.2%)
Bacillus	11(27.5%)	7(17.5%)	12(30.0%)	10(25.0%)	40(26.5%)
Lactobacillus	4(14.8%)	10(37.0%)	7(25.9%)	6(22.2%)	27(17.9%)
Staphylococcus	9(36.0%)	5(20.0%)	4(16.0%)	7(28.0%)	25(16.6%)
Escherichia	4(28.6%)	3(21.4%)	2(14.3%)	5(35.7%)	14(9.3%)
Streptococcus	-	-	-	2(100%)	2(1.3%)
Klebsiella	-	-	2(100%)	-	2(1.3%)
TOTAL	40(26.5%)	35(23.2%)	36(23.8%)	40(26.5%)	151(94.4%)

Table 5 shows the distribution of bacterial genera according to fried food types examined. Out of 151 (94.4%) positive samples, the genus Diphtheroids was isolated from the highest number of foods with 41 (27.2%), of which Fried Yams had the highest number of positive samples, followed by Fried Potatoes and Fried Plantain, Akara was the least with growth on 9 (22.0%) samples. Of the 40 (26.5%) samples that yielded Bacillus, Akara yielded growth of this organism in 12 (30.0%), followed by Fried Yams 11 (27.5%) and then Fried Plantain 10 (25.0%), Potatoes had the least of 7 (17.5%). Genus Lactobacillus was isolated from 27 (17.9%) samples, 10 samples of Fried Potatoes yielded growth of this organism, Akara 7 (25.9%), Plantain 6 (22.2%) and Yams were the least with 4 (14.8%). Genus Staphylococcus was isolated from 25 (16.6%) samples, of which Fried Yams had the highest number 9 (36.0%), Fried Plantain 7 (28.0%), Fried Potatoes 5 (20.0%) and Akara was the least with 4 (16.0%). Genus Escherichia coli was obtained from 14 (9.3%) samples, of which 5 (35.7%) samples of fried plantain yielded positive isolates, followed by Fried Yams 4 (28.6%), then Fried Potatoes 3 (21.4%) and Akara was the least 2 (14.3%). Fried Plantain and Akara yielded Streptococcus and Klebsiella in 2 (100%) samples each. The genus Streptococcus and Klebsiella 2 (1.3%) were the least isolates in the total of 151 (94.4%) positive samples and were recovered in Fried Plantain and Akara respectively. Statistically, there was no significant difference in the number of genus recovered from the various food types examined using ANOVA ($P > 0.05$).

Table 6:
Distribution Of Bacterial Isolates According To Period Of Sampling

FOOD TYPES	PERIOD SAMPLED		
	MORNING	EVENING	TOTAL
A (YAM)	25 (62.5%)	15 (37.5%)	40 (26.5%)
B (POTATO)	20 (57.1%)	15 (42.9%)	35(23.2%)
C (AKARA)	21 (58.3%)	15 (41.7%)	36 (23.8%)
D (PLANTAIN)	25 (62.5%)	15 (37.5%)	40 (26.5%)
TOTAL	91 (60.3%)	60 (39.7%)	151 (94.4%)

The distribution of bacterial isolates according to the period of sampling (morning and evening). Out of 151 positive samples, 91 (60.3%) food samples that yielded bacterial organisms were from morning samples while 60 (39.7%) were from evening food samples. The difference between the total number of isolates in the 2 periods was statistically significant using student T-test ($P < 0.05$). (Table 4.5)

Table 7:
Distribution Of Bacterial Isolates According To Zones

FOOD TYPES	ASATA	ABAKPA	GARIKI	OBIAGU	NEWHAVEN	TOTAL
A	8 (20.0%)	8 (20.0%)	8 (20.0%)	8 (20.0%)	8 (20.0%)	40 (26.5%)
B	8 (22.9%)	7 (20.0%)	7 (20.0%)	7 (20.0%)	6 (17.1%)	35 (23.2%)
C	8 (22.2%)	8 (22.2%)	7 (19.4%)	7 (19.4%)	6 (16.7%)	36 (23.8%)
D	8 (20.0%)	8 (20.0%)	8 (20.0%)	8 (20.0%)	8 (20.0%)	40 (26.5%)
TOTAL	32(21.2%)	31(20.5%)	30(19.9%)	30(19.9%)	28(18.5%)	151(94.4%)

KEY: A-YAM
B-POTATO
C-AKARA
D-PLATAIN

Table 7 shows the distribution of bacterial isolates according to zones. Of the 151 positive samples, Asata zone had the highest contaminated food samples with 32 (21.2%), of which all the food types sampled (Fried Yams, Fried Potatoes, Akara and Fried Plantain) yielded bacterial organisms, followed by Abakpa with 31 (20.5%) of which Fried Yams, Akara and Plantain yielded bacterial growth in all, Fried Potatoes yielded growth in 7 (20.0%) with no bacterial growth in one. Gariki zone had 30 (19.9%) of which Fried Yams and Plantain yielded bacterial growth in all food samples, Fried Potatoes and Akara yielded growth in 7 samples each. In Obiagu zone 30 (19.9%) food samples yielded bacteria organisms, out of which Fried Yams and Plantain yielded growth in all the 8 (20.0%) samples each, Fried Potatoes and Akara yielded growth in 7 samples each. In Newhaven 28 (18.5%) yielded bacteria organisms, Fried Yams and Plantain yielded growth in all the 8 (20.0%) samples, Fried Potatoes and Akara also yielded growth in 6 samples each. Statistically, there was no significant difference in the number of bacterial isolates from various zones using ANOVA ($P > 0.05$).

Table 8:
Distribution Of Bacterial Isolates According To The Source Of Fried Foods Examined

FOOD TYPES	COVERED	UNCOVERED	TOTAL
YAM	8 (20.0%)	32 (80.0%)	40 (26.5%)
POTATO	11 (31.4%)	24 (68.6%)	35 (23.2%)
AKARA	12 (33.3%)	24 (66.7%)	36 (23.8%)
PLANTAIN	16 (40.0%)	24 (60.0%)	40 (26.5%)
TOTAL	47 (31.1%)	104 (68.9%)	151(94.4%)

Table 8 shows the distribution of bacterial isolates according to the source of fried foods examined (covered and uncovered). Out of 151 samples that yielded positive isolates, 47 (31.1%) positive samples were from covered

food samples while 104 (68.9%) were from uncovered food samples. Statistically, there was significant difference in the number of samples that yielded bacterial organisms according to covered and uncovered using the T –test (P<0.05).

Table 9:
Contamination By Gram Negative And Gram Positive Bacteria According To Food Types

FOOD TYPES	GRAM POSITIVES	GRAM NEGATIVES	TOTAL
YAM	36 (90.0%)	4 (10.0%)	40 (26.5%)
POTATO	32 (91.4%)	3 (8.6%)	35 (23.2%)
AKARA	32 (88.9%)	4 (11.1%)	36 (23.8%)
PLANTAIN	35 (87.5%)	5 (12.5%)	40 (26.5%)
TOTAL	135 (89.4%)	16 (10.6%)	151(94.4%)

Table 9 shows the rate of contamination by Gram positive and Gram negative bacteria. Of the 160 food samples analyzed, 151 yielded bacterial growths, 135 (89.4%) being Gram positive organisms and the remaining 16 (10.6%) were Gram negative organisms. Fried Yams and Plantain yielded bacterial isolates in all the 40 (26.5%) samples, of which Gram positive organisms were obtained from 36 (90.0%) and 35 (87.5%) samples respectively, and the remaining 4 (10.0%) and 5 (12.5%) yielded Gram negative organisms respectively. Fried Potatoes had bacterial isolates in 35 (23.2%) samples, of which 32 (91.4%) were Gram positive organisms, while 3 (8.6%) were Gram negative organisms. Akara had bacteria growth in 36 (23.8%) samples, Gram positive organisms encountered were isolated from 32 (88.9%) samples and 4 (11.1%) samples yielded Gram negative organisms. The difference between Gram positive and Gram negative organisms encountered in the study was statistically significant using T-test (P<0.05).

Table 10:
Distribution Of All Bacterial Isolates According To Type (Mixed Growth)

FOOD TYPES	GRAM POSITIVES	GRAM NEGATIVES	TOTAL
YAM	45 (88.2%)	6 (11.8%)	51 (27.6%)
POTATO	40 (93.0%)	3 (7.0%)	43 (23.2%)
AKARA	44 (90.0%)	5 (10.2%)	49 (26.5%)
PLANTAIN	37 (88.1%)	5 (11.9%)	42 (22.7%)
TOTAL	166 (89.7%)	19 (10.3%)	185

The table indicates the distribution of all bacterial isolates according to type (Gram Positive and Gram Negative), including the mixed growth. Of the 151 contaminated food samples, 78 samples yielded pure growth while 73 were mixed growth, thus a total of 185 isolates were obtained. Of the 185 isolates, 166 (89.7%) were Gram Positive organisms while 19 (10.3%) were Gram Negative organisms. Of the 166 Gram Positive organisms, 45 were isolated from fried Yams, 40 from fried Potatoes, 44 from Akara, and fried Plantain were 37. Gram Negative organisms which were 19 (10.3%), yielded growth of 6 organisms in fried Yams, 3 in fried Potatoes and 5 in both Akara and Plantain. Statistically, there was significant difference between the Gram Positive and Gram Negative organisms isolated in the study (P<0.05).
(Table 4.8b)

Table 11:
Bacterial Counts From Four (4) Selected Food Types Sold In Enugu

FOOD TYPES	NO. OF FOODS EXAMINED	NO. CONTAMINATED	MEAN BACTERIAL COUNT (cfu/ml)	% CONTAMINATED
YAM	40	40	2.8 x 10 ²	100%
POTATO	40	35	2.0 x 10 ²	87.5%
AKARA	40	36	1.2 x 10 ²	90.0%
PLANTAIN	40	40	1.0 x 10 ²	100%

Table 11 shows the mean bacteria counts of the four (4) selected food types. The highest bacterial count of 2.8 x 10²cfu/ml together with the highest contamination rate of 100% occurred in fried Yams, followed by fried Potatoes with the mean bacterial count of 2.0 x 10²cfu/ml and contamination rate of 87.5%, Akara also gave mean count of 1.2 x 10²cfu/ml with contamination rate of 90.0%, then fried Plantain had the lowest mean

count of 1.0×10^2 cfu/ml with equivalent contamination rate of 100% to fried Yams. Statistically, there was significant difference in the bacterial counts of the different food types and also in the percentage contamination ($P < 0.05$).

IV. Discussion

Pathogenic bacteria are the most common known causes of food contamination and food borne illnesses. This study therefore aimed to analyze the bacteriological profile of fried ready-to-eat foods sold in various areas of Enugu and its environs.

Of the 160 randomly selected food samples examined, 151 (94.4%) showed bacterial growth, from which a total of nine bacterial species were identified. Nine of the 160 samples showed no bacterial growth, 135 (89.4%) of the 151 (94.4%) contaminated, yielded Gram Positive organisms while 16 (10.6%) were Gram Negative which when tested statistically was significantly different ($P < 0.05$). Salmonella and Shigella were also tested for in the study but not detected. The bacterial genera in decreasing order of occurrence are Diphtheroids 41 (27.2%), Bacillus 40 (26.5%), Lactobacillus 27 (17.9%), Staphylococcus 25 (16.6%), Escherichia 14 (9.3%), Streptococcus and Klebsiella 2 (1.3%) each, (table 4.2).

Among the bacteria isolated, the Genus Diphtheroids ranked highest with (27.2%). This organism has not been isolated in any food study and the isolation here could be as a result of environmental conditions. Diphtheroids are common commensals of the nose, throat, nasopharynx, skin, urinary tract and conjunctiva, and are generally unable to produce exotoxin, but a few cause disease in rare circumstances such as in immunosuppressed individuals. For example, Corynform ulcerans is sensitive to beta phage, and produces small amount of diphtheria toxin if lysogenized; it has been implicated in a mild diphtheria-like illness. Several species of Diphtheroids have been recovered in infections, such as endocarditis of prosthetic valves, lung abscesses, and urinary tract infections. Most strains have been shown to be multiple drug resistant Strohl et al. (2001), and should not be neglected when isolated in food.

Bacillus was isolated in (26.5%). This disagrees with the work done by Okonko, et al (2009) in Nigeria on hazards analysis critical control points and microbiology quality of sea foods as affected by handlers hygiene where Bacillus recorded only 24 (14.0%). The reason for this may be attributed to the type of foods used in the different studies. Members of the Genus Bacillus are ubiquitous in nature, as such can found their way to contaminate the food. The name Bacillus subtilis was often used to mean any aerobic, endospore-forming organism, but since 1970 there have been reports of infection in which identification of this species appears to have been made accurately. Cases associated with neoplastic disease include: fatal pneumonia and bacteremia, a septicemia, and an infection of a Necrotic axillary tumor in breast cancer patients. Breast prosthesis and ventriculo-atrial shunt infections, endocarditis in a drug abuser, meningitis following a head injury, cholangitis associated with kidney and liver disease, and isolations from surgical wound-drainage sites have also been reported. A

probiotic preparation of this species led to a fatal septicemia in an immunocompromised patient [26], [27], [28], [29] and [30]. Bacillus subtilis has also been implicated in food-borne illness, and a cases of bovine mastitis and of ovine abortion [31], [32], [33] and [34]. Bacillus subtilis has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions and can survive the extreme heat during cooking.

Bacillus cereus isolated in the study may be one of a major health hazard to consumers. It can cause food poisons due to its ability to produce enterotoxins and emetic toxins. Bacillus cereus spores can survive normal cooking procedures. Under improper storage conditions after cooking, the spores germinate and the vegetative cells multiply and can cause diarrheal illness. Also, it's been associated with nongastrointestinal infection, endophthalmitis and keratitis with abscess formation. Also documented as a cause of meningitis, septicemia, endocarditis, osteomyelitis etc. Few reports exist as B. cereus carrying B. anthracis toxin genes and hence can cause severe pneumonia clinically similar to pulmonary anthrax [35]. The widespread distribution of the organism and the ability of the spores to survive dried storage along with their thermal resistance, means that most ready-to-eat foods probably contain Bacillus cereus and will require control measures to prevent its growth [36].

Members of Genus Lactobacillus are widely distributed as saprophytes in vegetation and animal materials, or may be common human and animal commensals. They have complex nutritional requirement and obtain energy solely by the fermentation of carbohydrates. Their isolation from these foods can therefore be ascribed to this environment that favors them nutritionally. Lactobacillus has occasionally been associated with dental carriers and sub acute bacteria endocarditis [37].

Genus Staphylococcus was isolated in 25 (16.6%). This result however differs from that reported by Steven Tauro et al., on microbiological hazard identification and exposure assessment of food where

Staphylococcus aureus alone recorded 80 (61%). This may be attributed to environmental differences. *Staphylococcus aureus* and Coagulase negative *Staphylococci* are most often found on soil and on the mucous membrane. Contamination with *Staphylococcus aureus* might be direct during sales or unhygienic handling of the food during processing or due to contamination from the skin, mouth, or nose of the handlers which can be introduced directly into foods. *Staphylococcus aureus* are responsible for many pyogenic infection of man. It is also carried by 20-45% of healthy individual at any one time. *Staphylococcus aureus* causes immediate food poison because it releases preformed toxin that can establish immediately within few hours or minutes. There may not be any bacteria on examination but will keep causing infection due to the enterotoxin already formed which might not be destroyed by cooking or boiling.

The Coagulase Negative *Staphylococci* is universal skin commensals. The species of this organism are important pathogens of plastic devices, implanted metal and prostheses. This organism can cause urinary tract infection in sexually active women but have been associated with endocarditis and other systemic infections [38]. The Coagulase Negative *Staphylococci* normally present on the body surfaces as resident skin flora can reach the food by direct contamination from the hand of the workers or by dissemination via air and dust. They can also be found in the gut and upper respiratory tract.

Members of Enterobacteriaceae encountered in the study, including *Escherichia coli* and *Klebsiella aerogenes* can be attributed to environmental contamination by unwholesome materials like faeces. In a report by Bukar et al.(2009), 5 (10.0%) of 50 food handlers in three small-scale food industries in Kano Metropolis investigated carried *E.coli* on their hands. This percentage could easily cross-contaminate a whole production batch unnoticed. Ironically, most food handlers do not practice good personal hygiene and do not follow good manufacturing practices, which could reduce the occurrence of such bacteria in foods (Bukar et al.,2009; Kawo and Abdulmumin, 2009). Moreover, the faecal coliforms as *Escherichia coli* are generally considered as indisputable indicators of faecal contamination from warm blooded animals.

Streptococcus faecalis (*Enterococcus faecalis*) which is one of the least isolates normally colonize oral mucous membranes and skin of human and animal gut, and have been associated with urinary, biliary tract, abdominal wound infections and endocarditis. They can possibly find their way into the food through contamination by human and animal faeces mostly through contaminated water, dirty environment and also poor hygienic practices. They are also highly resistant to environmental and chemical agents and can persist in formites for a long period.

Results of this study showed that fried foods purchased from the uncovered source being contaminated with bacteria isolates in 104 (68.9%) and that purchased from covered source with 47 (31.1%), and there was statistically significant difference between the fried foods contamination according to their sources ($P<0.05$). It is not unlikely that foods prepared in our locality could be contaminated especially when not covered. Air borne microorganisms can contaminate foods during the point of selling. Also, some microorganisms on the surface of the skin, mouth, nose of the handlers can be introduced directly into the foods by process line workers, with lesions caused by *Staphylococcus aureus* on hands and arms coming in contact with the food, or by coughing and sneezing (Okonko et al.,2008; Sobuko et al.,2009).

The results also indicated that gram positive organisms isolated were very high, this could be ascribed to the fact that most Gram positive organisms isolated in this study are environmental organisms both in the soil and on the skin and can survive for longer period of time.

The result obtained in the distribution of the bacterial viable counts in terms of fried food types used. Yam had the highest contamination rate of 100% with the bacteria count of 2.8×10^2 cfu/ml while plantain had the lowest mean count of 1.0×10^2 cfu/ml with equivalent contamination rate 100% to fried yams. Statistically, there was significant variation ($P<0.05$) in both the bacteria count and percentage contamination according to the various food types. This could be due to the different food types used, as most bacteria ferment carbohydrate.

V. Conclusion

Health hazards from street food vending may be minimized by avoiding poor handling and awareness of need for personal hygiene and care in preparation, storage and dispensing of street foods. It has become necessary that systems should be put in place to ensure that food handlers remain aware of all procedures necessary to maintain the safety and suitability of food. Basic training in food hygiene is recommended to ensure that food vendors follow the required rules for proper hygiene and sanitation. Training on hygiene and sanitation, provision of continuous food safety education, screening of food handlers on regular basis for carriers, the establishment of code of practice for the street food industry and provision of basic water and waste management utilities are recommended to diminish the gap between knowledge and practices of safe street food vending.

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