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Antibiotic Resistance and Plasmid Curing Analysis of Bacteria Isolated from Street Vended Food in Owerri, Nigeria

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Abstract

Street vended foods are common in Nigeria, serving the purposes of supplementing family income and meeting food demands of low income urban dwellers. However, these foods can become a vehicle for transmitting food-borne pathogens due to poor processing and handling. This study aimed at assessing the safety, with respect to pathogenic bacteria, of three foods ['abacha' (African salad), roasted pork and 'ukwa' (*Treculia africana)*] commonly vended in Owerri, Imo state. Twelve samples [four each of 'abacha', roasted pork and 'ukwa'] were purchased from different vendors in Owerri metropolis, Imo state. Pathogenic bacteria were isolated from the food samples by the pour plate method on Nutrient, MacConkey, Eosin Methylene Blue and *Salmonella Shigella* agar plates and characterized using a combination of cultural/biochemical and 16S rRNA sequencing techniques. The antibiotic susceptibility test was performed for each isolate by the disk diffusion method. The plasmid profiles of isolates resistant to four or more antibiotics were determined followed by plasmid curing and a repeat of the antibiotic susceptibility test to indicate whether resistance is chromosomal or carried on a mobile element. Thirty-one isolates comprising seven species [*Acinetobacter baumanii* (KY417134), *Bacillus* sp., *Enterobacter sp*, *Escherichia coli (*KY417135), *Klebsiella* sp., *Kurthia gibsonii* (KY417133) and *Salmonella* sp.] were obtained from the food samples; 'ukwa' showed the highest microbial load. Isolates (96.8%) showed the highest resistance against nalidixic acid and ampicillin. Multi drug resistance was observed in 11 (35.5%) of the isolates, seven of which harboured plasmids ranging 0.5 - 48.5 kb. Plasmid curing improved sensitivity of isolates to all antibiotics except ampicillin and nalidixic acid.

Keywords: *Safety; Antibiotics; Plasmids; Curing; Resistant; Susceptible*

Introduction

Street foods are described as ready-to-eat foods and beverages prepared and sold by vendors or hawkers especially in streets and other similar public places [1]. Street foods contribute significantly to the diets of many people in the developing world Moreover, street foods play an important role in developing societies as they support the livelihoods of millions of the urban poor. In Nigeria, the presence of street foods and street food vendors has become one of the most fascinating aspects of social life in urban and semi-urban centres. In fact, street food trade in Nigeria is becoming a viable and important informal-sector industry. In addition to enhancing the income of operators, street foods helps in meeting the food demands of urban dwellers especially the low income group [2,3].

However, street foods have in recent years become one of the most common risks associated with the increase in outbreaks of food-borne diseases in developing countries. Street foods are perceived to be a major public health risk due to lack of basic infrastructure and services, difficulty in controlling the large numbers of street food vending operations because of their diversity, mobility and temporary nature [4-6]. Microbiological studies from many developing countries, carried out on street vended food have revealed a high bacte-

ria count. *Salmonella* species, *S. aureus* and members of the family Enterobacteriaceae were common pathogens found in such food items [7-9]. In Nigeria, surveys carried out by the department of Public Health of the Federal Ministry of Health in 2006 and 2007 gave over two million cases of food borne diseases and the number of death put at over five hundred thousand. Many of these deaths were linked to the consumption of contaminated street vended foods. Also, the Consumer Protection Council survey on street vended foods within the Federal Capital Territory Abuja gave a similar scenario that presupposes that the situation could be worse in the hinterland [10].

In addition, the prevalence of multi-drug resistance among important microorganisms, such as *Salmonella, E. coli,* and *S. aureus* has been increasing and poses a real threat to public health [11,12] because street foods could possibly become a medium by which these antimicrobial-resistant pathogenic bacteria are transmitted to people.

Bread fruit ('Ukwa'), African salad (Abacha) and roasted pork meat are amongst the most commonly vended foods in Owerri, Imo state Nigeria. Bread fruit (*Treculia Africana*) is an important food crop in Nigeria. It is usually cultivated in the southern states of Nigeria [13,14]. The plant produces large, usually round compound fruit covered with rough pointed outgrowths. The seeds are buried in spongy pulp of the fruit [15,16]. The seeds are variously cooked as porridge alone or mixed with other food stuff such as sorghum [17] or roasted and sold with palm kernel (*Elaeis guineensis*) as a roadside snack. The flour has high potential usage for pastries [18]. The seeds are highly nutritious and constitute a cheap source of vitamins, minerals, proteins, carbohydrates and fats [16]. Abacha, is an exotic delicacy and a special recipe native to Nigeria. Abacha is widely accessed for its composition of food ingredients known to be rich in protein, carbohydrate, vitamins, and minerals. It can be consumed on its own or in combination with other snacks like coconut, palm kernel and groundnut. Abacha is usually eaten as an in-between meal or as a side dish to the various Nigerian rice recipes [19,20]. Abacha is processed by harvesting cassava tubers, after which they are peeled, washed and boiled. These are then shredded into fine slices, and soaked for 24 hours for fermentation to occur so as to thoroughly reduce the starch and hydrogen cyanide from the cassava. The shredded and fermented cassava is again thoroughly washed the following day before drying it for 2 - 3 days [21]. Pork which is obtained from swine serves as food. It is a major source of protein and fats, and also an important source of vitamins for most people in many parts of the world. The pork is washed, Seasoned with salt, pepper and garlic powder and roasted. These pork based street foods are usually sold in many public places such as bus terminals, parks and are prone to infection with microorganism and may pose serious health hazard to the final consumer. This study was therefore undertaken to isolate and characterize bacterial specie from the three selected vended foods using 16SRNA sequencing. The result may provide information on the microbial hazards present in these street-vended foods and can be used as inputs to microbiological risk assessments. The study also evaluated the antibacterial resistance profile, plasmid analysis and curing of the resulting microbial isolates. The results of which may offer valuable information on the potential of street foods to contribute to the spread of multidrug-resistant microorganisms.

Materials and Methods

Street-vended food consisting of bread fruit ('ukwa"), "abacha" and pork samples were used in this study. Four samples were purchased from Douglas road, Amakohia road and worldbank road all in Owerri meteropolis. Each sample consisted of approximately 100g, and the samples were collected from the point-of-sale in packages provided by the vendors, just as a consumer would do. The packed samples were placed in a cooler and immediately transported to the laboratory and stored at 4°C until they were analyzed; the holding time did not exceed 16h.

Isolation and Enumeration of Bacteria

Ten (10g) of each food samples were homogenised with sterile mortar and pestle, the resulting homogenate were aseptically added to 9 ml sterile normal saline and serially diluted up to 10^{-5} using 0.1% peptone water. 0.1 ml of the various dilutions were inoculated into sterile nutrient agar, MacConkey agar, Eosin methylene agar and *Salmonella Shigella* agar using pour plate method. The plates were incubated

at 37°C for 24 hours for bacterial isolates after which the colonies were counted. Single or discrete colonies were streaked out to get pure culture. Isolates obtained were purified by a two-time repeated streaking on fresh agar plates of tryptone soy agar and maintained on the same agar slants at 4°C at the Microbiology Laboratory of Imo State University

Identification of bacterial isolates

Morphological and biochemical identification

For preliminary identification of isolates to genus level, cell morphology (Gram reaction, cell shape and arrangement) were carried out. All 31 selected colonies were examined under a microscope after Gram staining. Simple biochemical tests (e.g. catalase, citrate, oxidase, motility, indole and carbohydrate utilization was carried out. All isolates were preserved in tryptone soy agar stab culture (LAB M, UK) and sent to macrogen incorporated Europe for molecular characterization.

Antibiotic susceptibility testing

The disk diffusion method of Bauer., *et al.* [22] was used to determine the **antibiotic resistance** profile of isolates against the following antibiotics; tarivid (OFX) 10 µg; ciproflox (CPX) 10 µg; gentamicin (AU) 30 µg; Gentamycin (CN) 10 µg; Streptomycin (S) 30 µg; Ceporex (CEP) 10 µg; Nalidixic acid (NA) 30 µg, Septrin (SXT) 30 µg and Ampicillin (PN) 30µg. The disks were purchased from Optun Laboratories Nigeria. Pure cultures of **each** isolates were grown overnight in Tryptic Soy Broth (TSB) (Oxoid Limited, Basingstoke, UK) at 37°C and the concentration adjusted using sterile TSB until a 0.5 McFarland turbidity was attained. 100ml of the culture was then swabbed onto Mueller Hinton agar (Oxoid Limited, Basingstoke, UK) using a sterile cotton swab. Three antimicrobial disks were placed on the surface of the agar plate at a distance to avoid overlapping of inhibition zones. The plates were incubated at 37°C for 16 - 18h and the results were interpreted as sensitive, intermediate, or resistance according to Clinical and Laboratory Standards Institute guidelines for CLSI [23].

Plasmid profile

Detection of plasmids among antibiotic-resistant isolates

Plasmid analysis was performed on representative isolates selected on the basis of their antibiotic resistance phenotypes. All isolates selected for plasmid analysis were multiple resistant isolates (resistant to at least 9 of the antibiotics used) with diverse phenotypes.

DNA isolation and plasmid profile

Organisms were grown overnight in Luria-Bertani (LB) broth at 37°C with aeration using an orbital shaker and plasmid DNA was extracted from lysed isolates using Plasmid Miniprep kit from Promega Corporation (USA).

Agarose gel electrophoresis of plasmid DNA

Electrophoresis was carried out in a horizontal gel apparatus (Scie-Plas limited, Southam, Warwickshire, United Kingdom). Electrophoresis was conducted in agarose (0.8%) gel (Inqaba Biotech., South Africa) and stained with ethidium bromide. The approximate molecular mass of plasmids (in mega daltons) was determined by comparing with Lambda DNA Hind III digest (Promega-USA) as a standard marker

Plasmid curing

Curing of plasmid was done [24] by exposing the overnight grown culture to elevated temperature (37ºC) and 1% Sodium Dodecyl Sulphate (SDS). These cultures were then streaked onto Nutrient agar plates and incubated for 24h. The colonies found were cured colonies and was inoculated to sterile Nutrient broth. Antibiotic sensitivity profile of cured colonies on Mueller Hilton agar was done using the previous antibiotic discs.

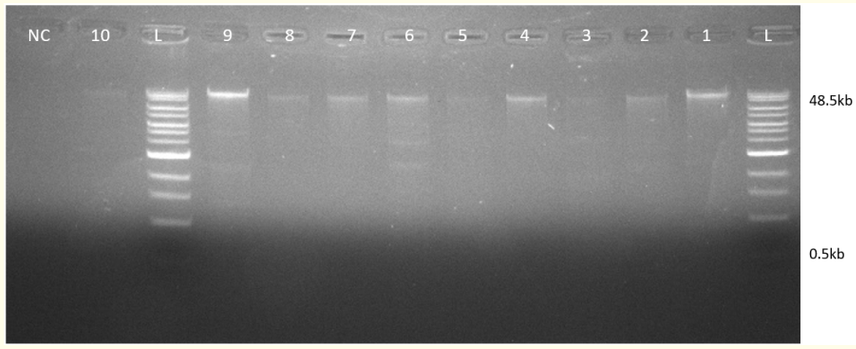

Figure 1: *Plasmid profile of multiple drug resistance bacteria isolates analyzed with 0.8% agarose gel electrophoresis stained with ethidium bromide. L is 0.5kb - 48.5 kb DNA ladder (molecular marker). Samples 1 and 9 are positive for plasmid genes with bands 48.5 kb, samples 2, 4, 6, 7 and 8 are positive for plasmid genes with bands 45 kb while samples 3, 5 and 10 are negative for plasmid genes. NC is a no plasmid DNA template control.*

Results and Discussion

Isolation and enumeration of bacterial from vended food samples

The result of the colony count of the food samples is shown in table 1. It was observed that total viable count (9.5 X 10^5 cfu/g) was highest with "ukwa" samples purchased from Amakohia road while pork samples from world bank road had the lowest viable count of 7.0 X10^4cfu/g. Total coliform count was highest with ukwa samples obtained from world bank street while pork samples from Douglas road had the lowest count (6.0 X 10^4 cfu/g). Pork samples purchased from Amakohia road had the highest feacal coliform count while "ukwa" samples purchased from World Bank had the lowest feacal coliform count. Results also showed that SSB counts were highest with "abacha" samples purchased from Douglas road and lowest with "ukwa" samples from Amakohia road (2.6 X 10^5 cfu/g). These values fall within the unacceptable limit for microbial quality standard of ready to eat food (ICMSF, 1994; FAO/WHO, 2005). These high counts could be attributed to the poor hygienic conditions of the vendors, the environment and raw materials used for the cooking. Several researchers have also reported high bacteria counts in street vended foods in Nigeria [2,4]. Abacha is a product consumed raw with no heat treatment to reduce microbial load. This could be associated with the high bacteria counts associated with the product (Oranusi., *et al*. 2013). In addition, ingredients such as Ugba and Ogiri are usually added to abacha during the preparations. These two products could also contribute to the high microbial load (Nwagu., *et al.* 2010; Obeta 1983; Isu and Njoku, 1997). The high feacal counts recorded in pork samples showed the microbial diversity in this location, condition of the location and hygienic practice employed by the pork vendors. It could be an indication of re-contamination and hygiene techniques (Clarence., *et al.* 2009). A total of 31 isolates (10 from abacha, 11 from pork meat and 10 from ukwa) were isolated from the three vended food samples. Preliminary identification based on cultural and biochemical characteristics, classified isolates into seven species which include *Bacillus cereus, Enterobacter spp, Escherichia coli, Klebsiella pneumoniae, Kurthia gibsonii, Acinetobacter baumanii and Staphylococcus* (Table 2) *E. coli (*KY417135) was the most predominant organism with a frequency of (22.58%), followed by *Bacillus cereus* (19.35%) while *Kurthia gibsonii* (6.45%) and *Klebsiella* spp (6.45%) had the least frequency of occurrence (Table 3). Isolates were confirmed using 16sr RNA sequencing. *Bacillus cereus*, *Salmonella* spp and *E. coli* were implicated in all three samples while *Klebsiella pneumoniae* and *Enterobacter* spp were isolated from "abacha" and pork. However, *Kurthia gibsonii* (KY417133) and *Acinetobacter baumanii (*KY417134) were isolated from pork and "ukwa" respectively. *Bacillus* was isolated from each of the vended food samples (Table 4). Microorganisms isolated from these food samples in this study have been implicated in foods, their pattern is similar to previous reports.

Food samples	Sample location	Nutrient agar	MacConkey	EMB	SSA
Pork	Douglas road	3.5 X 10^5	6.0 X 10^4	5 X 10^4	4.2 X 10^5
Pork	Amakohia road	2.9 X 10^5	1.3 X 10^5	3.0 X 10^4	3.3 X 10^5
Pork	World bank road	7.0 X 10^4	9.5 X 10^4	7 X 10^4	3.8 X 10^5
Abacha	Douglas road	5.3 X 10^5	6.9 X 10^5	1.1 X 10^6	1.9 X 10^6
Abacha	Amakohia night joint	7.7 X 10^5	7.2 X 10^5	8.9 X 10^5	8.7 X 10^5
Abacha	World bank road	1.5 X 10^6	8.2 X 10^5	3.3 X 10^5	9.6 X 10^5
Ukwa	Douglas road	1.7 X 10^6	6.9 X 10^5	1.72 X 10^6	5.5 X 10^5
Ukwa	Amakohia road	9.5 X 10^5	1.76 X 10^5	9.5 X 10^5	2.6 X 10^5
Ukwa	World bank road	8.9 X 10^5	2.7 X 10^6	1.76 X 10^6	3.3 X 10^5

Table 1: *Mean count of bacteria isolated from selected vended foods cfu/g.*

Isolates	Food sample	OFX	PEF	CPX	AU	CN	S	CEP	NA	SXT	PN
1	A	S	S	S	S	S	I	S	S	S	R
2	P	R	R	R	R	S	R	R	R	R	R
3	P	R	R	S	S	R	S	R	R	R	R
4	P	R	R	R	R	R	R	R	R	R	R
5	P	S	S	R	R	S	S	S	I	I	S
6	P	R	R	R	I	R	R	R	R	R	R
7	P	S	S	R	I	S	I	I	R	R	I
8	P	S	S	R	S	R	I	S	R	R	I
9	P	S	R	S	R	I	I	S	R	R	I
10	P	I	R	R	I	I	I	I	R	I	I
11	P	I	I	S	I	I	S	I	R	S	S
12	A	R	R	R	R	R	I	R	R	R	R
13	A	R	R	R	R	R	I	R	R	R	R
14	A	R	I	S	I	R	S	I	R	S	R
15	A	R	R	S	S	R	S	R	R	S	R
16	A	R	R	S	R	R	S	R	R	S	R
17	A	R	R	R	R	R	S	R	R	S	R
18	A	I	R	R	R	R	R	R	I	R	R
19	A	R	R	I	R	R	R	R	R	I	I
20	A	R	R	R	R	R	R	R	R	R	R
21	U	R	R	I	R	R	I	S	R	R	R
22	U	I	R	R	R	R	I	R	R	R	I
23	U	R	R	R	R	I	R	R	R	R	R
24	U	R	R	R	R	R	I	R	R	R	R
25	U	I	R	R	R	R	I	I	R	R	I
26	U	R	R	R	R	R	R	R	R	R	R
27	U	R	R	R	R	R	R	R	R	R	R
28	U	R	R	I	R	R	R	R	R	R	R
29	U	R	R	R	R	I	I	R	R	R	I
30	U	I	R	R	R	I	R	R	R	R	R
31	U	I	R	R	R	I	R	R	R	R	R

Table 2: *Antibiotic profile of bacteria isolates before curing.*

Key: A: Abacha; P: Pork; U: Ukwa; R: Resistance; S: Sensitive; I: Intermediate. OFX: Ofloxacin (10mcg); PEF: Pefloxacin (10mcg); CPX: Ciprofloxacin (10mcg); AU: Augmentin (30mcg); CN: Gentamycin (10mcg); S: Streptomycin (30mcg); CEP: Ceporex (10mcg); NA: Nalidixic Acid (30mcg); SXT: Septrin (30mcg); PN: mpicillin (30mcg).

Bacillus cereus: 1, 2, 3, 16, 17, 29; Salmonella spp: 18, 10, 11, 25, 30; Klebsiella spp: 14, 15, 5, 22, 7; Kurthia gubsonii: 4, 23; Acinetobacter baumannii: 27, 26; Enterobacter spp: 8, 31, 9, 21; E. coli: 6, 12, 13, 20, 24, 28, 19.

Isolate	No of occurrence	Frequency (%)
Bacillus spp	6	19.35
Salmonella spp	5	16.13
Klebsiella spp	5	16.13
Kurthia gibsonii	2	6.45
Acinetobacter baumanii	2	6.45
Enterobacter spp	4	1230
E. coli	7	22.58

Table 3: *Percentage occurrence of each isolate in the food samples.*

S/NO	Organisms	OFX	PEF	CPX	AU	CN	S	CEP	NA	SXT	PN
1Pre	*Bacillus cereus*	R	R	R	R	R	R	R	R	R	R
Post		S	R	S	R	S	S	R	R	I	R
2 Pre	*Bacillus cereus*	R	R	R	R	S	R	R	R	R	R
Post		S	R	S	I	S	I	S	R	S	R
3 Pre	*Kurthia gibsonii*	R	R	R	R	R	R	R	R	R	R
Post		S	S	S	S	S	S	S	I	S	R
4 Pre	*Escherichia coli*	R	R	R	R	R	I	R	R	R	R
Post		I	R	S	R	S	S	R	R	R	R
5 Pre	*Enterobacter spp*	R	R	R	R	R	I	R	R	R	R
Post		S	S	S	S	S	S	S	R	S	I
6 Pre	*Klebsiella pnemoniae*	I	R	R	I	R	R	R	R	R	R
Post		S	S	S	I	S	S	R	I	S	I
7 Pre	*Enterobacter spp*	I	R	R	R	I	R	R	R	R	R
Post		S	S	R	S	I	S	R	S	I	R

Table 4: *Antibiotic profile of multi drug resistant isolates after curing.*

Key: Pre: Pre antibiogram; Post: Post antibiogram.

Oranuis., *et al.* (2013) reported the presence of *Bacillus spp, Staphylococci, Escherichia coli, Enterococci* and *Serratia* from ready to eat abacha samples. Nkechinyere (2010) implicated *E. coli, Streptococcus and Staphylococcus* from suya meat in Enugu State. In their study, Oranusi., *et al.* (2015) reported the prevalence of *S. aureus, B. cereus, P. aeruginosa, Klebsiella* spp. The presence of this organism indicates poor sanitary control practices. *Bacillus cereus* is associated with the production of toxin in food which causes food poisoning. It is found in dust, soil and raw food and can survive normal cooking as a heat resistant spore (Rajkowski and Bennett, 2003). The presence of *Staphylococcus aureus* in the samples is indicative of human contamination after production. This could be from direct human contact such as fingers or indirectly through additives or utensils. The organism is associated with endotoxin characterized by short incubation period (1 - 8 hours), violent nausea, vomiting and diarrhea. *E. coli, Salmonella, Enterobacter* and *Klebsiella* are members of Enterobacteriaceae and are mainly found in water, soil and feacal matter. The presence of these organisms in these street foods reveals a poor state of hygiene and sanitary practices adopted during the preparation and packaging of these street foods (Jay, 2005). Although some *E. coli* are harmless, Enterohaemorrhagic *E. coli* (EHEC) are capable of producing one or more toxin and a particular serotype O157:H7 have been associated with haemorrhagic colitis, haemolytic uraemic syndrome and thrombotic thrombocytopaenic purpura. Also Enterotoxigenic *E. coli* (ETEC) is associated with traveler's diarrhea. *Kurthia* species are widely distributed in the environment and are common in feces of farm animals, milk, soil and surface waters, meat and meat products after cold storage. The presence of these organisms may have been due to re -contamination and have been implicated as opportunistic pathogens reported to cause endocarditis (Murray., *et al.* 1995).

Antibiotic resistant profile of isolates showed that nalidixic acid (93.6%) and pefloxacin (80.7%) were the most resistant antibiotics. This was followed by ceporex (71%) while streptomycin had the least resistant (41.9%) (Table 4). Ten (32.25%) of isolates were resistant to four or more of the antibiotics. The high resistance of the isolates against the various antibiotics (especially the quinolones and fluroquinolones) used in this study may in part be due to various factors such as inappropriate usage of antibiotics and drug resistance mechanism possessed by the bacterial isolates. Ojo., *et al.* [25] reported prevalence of multi drug resistance of fluoroquinolones against *Staphylococci* species isolated from wounds and burns. Quinolones and fluoroquinolones are currently used to treat variety of human infections caused by both Gram-positive and Gram-negative bacteria due to their broad-spectrum antimicrobial activity. Their widespread and often indiscriminate use, however, has resulted in ubiquitous resistance, especially among members of the Enterobacteriaceae (Spellberg and Doi, 2015; Aldred., *et al.* 2014; Redgrave., *et al.* 2014; David., *et al.* 2014). Plasmid gene was detected in samples 1, 2, 4, 6, 7, 8 and 9 which represents 26.1% of the multidrug resistance strains of the isolates analyzed. *Bacillus cereus* isolated from Abacha and *Enterobacter aerogene* isolated from pork had plasmid genes with molecular weight of 48.5 kb while *Bacillus cereus, Kurthia gibsonii, Escherichia coli*, *Enterobacter aerogene* and *Klebsiella pneumonia* isolated from pork samples had plasmid genes with molecular weight of 45 kb. This agrees with the work of agreement with the work of Oleghe., *et al.* [26] who isolated resistance plasmids (R factor) from the organisms implicated in this work. Alam., *et al.* [27], Farshad., *et al.* [28] isolated plasmid DNA genes with molecular weight range of 0.5 - 33 kb from *E. coli.* Lyon., *et al.* [29] and Gillespie., *et al.* [30] isolated small plasmids with no attributable functions but probably responsible for important accessory functions not associated with resistance to any antimicrobial agents tested. Plasmid mediated resistance to various antibiotics have been demonstrated by various workers [31-37]. Plasmid curing improved sensitivity of isolates to some of antibiotics except ampicillin, nalidixic acid and ceporex. Resistance to streptomycin, gentamycin and ofloxacin was completely reversed. Resistance of isolates to ampicillin and nalidixic acid shown in his work could be chromosomal mediated and not plasmid.

Isolates curing	Resistance profile before curing	Resistance profile after
Bacillus cereus	OFX, PEF, CPX, AU, CN, S, CEP, NA, SXT, PN	PEF, AU, CEP, NA, SXT, PN
Bacillus cereus	OFX, PEF, CPX, AU, S, CEP, NA, SXT, PN	PEF, AU, S, NA, PN
E. coli	OFX, PEF, CPX, AU, CN, S, CEP, NA, SXT, PN	No plasmid
Kurthia gibsonii	OFX, PEF, CPX, AU, CN, S, CEP, NA, SXT, PN	CEP, NA, PN
Salmonella spp	OFX, PEF, CPX, AU, CN, S, CEP, NA, SXT, PN	No plasmid
E. coli	OFX, PEF, CPX, AU, CN, S, CEP, NA, SXT, PN	OFX, PEF, AU, S, NA, SXT, PN
Bacillus cereus	OFX, PEF, CPX, AU, CN, S, CEP, NA, SXT, PN	NA, PN
Klebsiella pneumoniae	OFX, PEF, CPX, AU, CN, S, CEP, NA, SXT, PN	AU, CEP, NA, PN
Enterobacter spp	OFX, PEF, CPX, AU, CN, S, CEP, NA, SXT, PN	CPX, CN, CEP, PN
Acinetobacter baumanii	OFX, PEF, CPX, AU, CN, S, CEP, NA, SXT, PN	No plasmid

Table 5: *Profile of multi drug resistance bacterial isolates before and after curing.*

Antibiotics	No. (%) resistance before curing	No. (%) resistance after curing
Ofloxacin	8 (80)	1 (10)
Pefloxacin	10 (100)	4 (40)
Ciprofloxacin	10 (100)	1 (10)
Augmentin	10 (100)	1 (10)
Gentamycin	9 (90)	1 (10)
Streptomycin	9 (90)	1 (10)
Ceporex	10 (100)	1 (10)
Nalidixic Acid	10 (100)	2 (20)
Septrin	10 (100)	1 (10)
Ampicillin	10 (100)	2 (20)

Table 6: *Percentage of multi drug resistant isolates before and after curing.*

Conclusion

The result of the study revealed the presence of bacteria in the street food samples with unacceptable microbiological limits. This work also implicated the presence of isolates which could pose serious health hazards to consumers. The study therefore recommends more vigorous public awareness and enlightenment on safe food preparation to protect the public from contacting the spread of disease pathogens.

Bibliography

1. FAO. "Street foods. A summary of FAO studies and other activities relating to street foods". Rome FAO (1989).
2. Metiboba S and Kakwagh VV. "Safety and Hygienic Implications of Street Vended Foods in Anyigba Town, Kogi State". *Nigeria Food Science and Quality Management* 34 (2014).
3. Oranusi S and Olorunfemi OJ. "Microbiological safety evaluation of street vended ready-to-eat fruits sold in Ota". *International Journal of Research in Biological Sciences* 1.3 (2011): 22-26.
4. Oranusi US and Braide WA. "Study of microbial safety of ready-to-eat foods vended on highways: Onitsha-Owerri, south east Nigeria". *International Research Journal of Microbiology* 3 (2012): 66-71.
5. De souza CP. "The impact of food manufacturing practices on foodborne diseases". *Brazilian Archives of Biology and Technology* 51.4 (2008): 815-8258.
6. Ghosh M., *et al*. "Prevalence of enterotoxigenic Staphylococcus aureus and Shigella spp. in some raw street vended Indian foods". *International Journal of Environment Health Research* 17.2 (2007): 151-157.
7. Buck JW., *et al*. "Recent trends in microbiological safety of fruits and vegetables". *Plant Health Progress* 10 (2003): 1094.
8. Mosupye FM and VonHoly A. "Microbiological quality and safety of ready-to-eat street-vended foods in Johannesburg, South Africa". *Journal of Food Protection* 62.11 (1999): 1278-1284.
9. Bryan F., *et al*. "Hazards associated with holding and reheating foods at vending sites in a small town in Zambia". *Journal of Food Protection* 60.4 (1997): 391-398.
10. Agu I. "Food safety practices in Nigeria". *Food Control* 20.1 (2011).
11. Guven K., *et al*. "Occurrence and characterization of Staphylococcus aureus isolated from meat and dairy product consumed in Turkey". *Journal of Food Safety* 30.1 (2010): 196-212.
12. Harakeh S., *et al*. "Isolation, molecular characterization and antimicrobial resistance of Salmonella and Escherichia coli isolates from meat-based fast food in Lebanon". *Science of the Total Environment* 341.1-5 (2005): 33-44.
13. Badifu GIO and Akubor PI. "Influence of PH and sodium chloride on selected functional and physical properties of African breadfruit (Treculia Africana Decen) kernel flour". *Plant Foods for Human Nutrition* 56.2 (2001): 105-115.
14. Ugwu FMFC., *et al*. "Protein quality indices and food intake pattern of parboiled and roasted breadfruit-corn diets". *Journal of the Science Agriculture Food and Technology Environment* 2 (2001): 97-100.
15. Keay RWJ. Trees of Nigeria: A Revised Version of Nigerian Trees volume 1 and 2 Oxford, Standfield D.P, Clarendon Press (1989).
16. Osuji JO and Owei SD. "Mitotic index studies on Treculia africana Decne in Nigeria". *Australasian Journal of Agricultural Engineering* 1.1 (2010): 25-28.
17. Onweluzo JC and Nnamuchi OM. "Production and Evaluation of porridge-Type Breakfast product from Treculia Africana and Sorghum Bicolor flours". *Pakistan Journal of Nutrition* 8.6 (2009): 731-736.
18. Onyekwelu JC and Fayose OJ. "Effect of storage methods on germination and proximate composition of Treculia africana seeds". Conference on International Agricultural Research for Development. University of Kassel-I-Witzen-hausen and University of Gottigen (2007).

19. Maky P. "Nigerian salad recipes-How to make your own" (2013).

20. Ihekoronye IA and Ngoddy PO. "Integrated food science and technology for the tropics". Macmillan Educ. Ltd, London and Oxford 1st edition (1985): 172-190, 298-352.

21. Ekwu FC., *et al*. "Abacha making quality of selected cassava variety". *Nigerian Food Journal* 27.1 (2009): 49-58.

22. Bauer., *et al*. "Antibiotic susceptibility testing by a standardized single disk method". *American Journal of Clinical Pathology* 45.4 (1966): 493-496.

23. CLSI. Clinical and Laboratory Standards Institute (CLSI). "Performance standards for antimicrobial susceptibility testing sixteen international supplements CLSI Document". Wayne, PA: CLSI (2006).

24. Fortina MG and Silva M. "Mapping of Two Plasmids from Lactobacillus helveticus ILC54, Plasmid Curing and Preliminary Studies on Their Involvement in Lactose Metabolism and Peptidase Activity". *Biotechnology Letters* 18.9 (1996): 1003-1006.

25. Ojo SKS., *et al*. "Plasmid curing analysis of antibiotic resistance in beta-lactamase producing Staphylococci from wounds and burns patients". *Pakistan Journal of Biological Sciences* 17.1 (2014): 130-133.

26. Oleghe PO., *et al*. "Multidrug resistance bacterial isolates recovered from herbal medicinal preparations in Southeastern setting, Nigeria". *Journal of Rural and Tropical Public Health* 10 (2011): 70-75.

27. Alam MJ., *et al*. "Antibiogram and Plasmid profiling of E. coli isolates". *International Journal of Biological Research* 1.3 (2010): 1-7.

28. Farshad S., *et al*. "Microbial Susceptibility, virulence Factors, and Plasmid profiles of Uropathogenic Escherichia coli strains isolated from children from Jahrom, Iran". *Archives of Iranian Medicine* 15.5 (2012): 312-316.

29. Lyon BR., *et al*. "Molecular epidemiology of multiresistant Staphylococcus aureus in Austrian Hospitals". *Journal of Medical Microbiology* 17 (1984): 79-89.

30. Gillespie MT., *et al*. "Antibiotic susceptibilities and plasmid profiles of nosocomial methicillin-resistant Staphylococcus aureus: a retrospective study". *Journal of Medical Microbiology* 17.3 (1984): 295-310.

31. Raghada SM., *et al*. "Detection of plasmid DNA profile in bacterial and fungal isolates from catheterizeds patients and its relation with antibiotic susceptibility". *Journal of Biotechnology Research Center* 7.2 (2013): 51-60.

32. Olufunmi and Inomiset. "Multidrug resistance among bacteria isolated from some foods sold in Restaurants in Abraka, Nigeria". *International Journal of Microbiological Research and Review* 2.6 (2013): 97-102.

33. Ehiaghe FA., *et al*. "Plasmid Profiling and Curing Analysis of Fluoroquinolone Multidrug Resistant Pseudomonas aeruginosa in Benin City, Nigeria". *Open Journal of Medical Microbiology* 3.2 (2013): 201-205.

34. Shadid M. "Plasmid mediated Amikacin Resistance in clinical isolates of Pseudomonas aeruginosa". *Indian Journal of Medical Microbiology* 22.3 (2004): 182-184.

35. Daini OA., *et al*. "Quinolones Resistance and R-Plasmids of Clinical Isolates of Pseudomonas species". *Sudan Journal of Medical Sciences* 3.2 (2008): 139-146.

36. Olayinka AT., *et al*. "Antibiotic Susceptibility and Plasmid pattern of Pseudomonas aeruginosa from the surgical unit of University Teaching Hospital in North Central, Nigeria". *International Journal of Medicine and Medical Sciences* 1.3 (2009): 79-83.

37. Birnboim HC and Doly J "Alkaline Extraction Procedure for Screening Recombinant Plasmid DNA". *Nucleic Acids Research* 7 (1979): 1513-1523.

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