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Bacterial composition of roasted plantains and maize sold in Ado-Ekiti main Motor Park and their antibiotics sensitivity

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Abstract

Problem: Ready to eat foods are as important as home prepared meals because both are for nutrient requirements but the manners of ready to eat food preparation and microbial contamination is worrisome. Roasted plantains and maize desired by many Nigerian and are sold all over the country as local snacks to breach the gap between meals or may also be taking by some persons as full meal. **Methodology:** To evaluate the microbiological quality of these snacks sold at Ado Ekiti main Motor Park, 12 samples each were purchased from vendors and processed under aseptic condition. **Finding:** Bacterial counts (2.73×10^5 - 67×10^3 CFU/g) and coliform counts (33×10^3 - 10×10^3 CFU/g) in the plantains sample were higher than the counts from roasted maize (2.14×10^5 - 65×10^3 CFU/g) and (29×10^3 - 2×10^3 CFU/g) respectively. *Bacillus cereus* was the most occurred bacteria among the isolates. *P. aeruginosa* was the most inhibited isolate by halo of 18 mm with cefuroxime and *K. earogenes* the least inhibited by halo of 1 mm with tetracycline and ofloxacin. **Conclusion:** Methods of displaying the snacks after preparation is unhygienic and could contribute immensely to the contamination of the products.

Keywords: 1 Local snacks, 2 antibiotics, 3 roasted, 4 bacteria, 5 vended, 6 Ado Ekiti

Introduction

Ready to eat foods have served a significant aspect of quick feeding to many Nigerian populace. Though some are expensive, the life style of many individuals depend on it for nutritional requirements.

Snacks in Western culture are the types of foods to assuage hunger between meals for quantity supply of body's energy (James, 2005). Vending of snacks is a helpful services to travelers, workers, artisans, school pupils and several other persons. The convenience in buying and consumption of snacks depends solely on interest and not the aspect of its quality and safety (WHO, 2011). A general observation of Ado Ekiti inhabitants shows a social life pattern where large percentage of the people depends on vended ready to eat foods.

Roasted plantain and corn (plates 1 and 2) normally displayed at road sides was envisaged to be high in microbial contaminants as result of dust from moving vehicles, passersby the producers and vendors with little or no knowledge on good hygiene practice. Vehicular emission has been implicated as major source of pollution of most street vended foods (Opeolu, 2010). Street food vending has become an important public health issue and a great concern to everybody due to widespread of food borne diseases from inadequate understanding of basic food safety measures (Rane, 2011). Major sources of microbial contamination are the place of preparation, utensils for cooking and serving, raw materials and personal hygiene of vendors.

Several research studies all over the world have listed *Staphylococcus*, *Salmonella*, *Bacillus*, *Clostridium*, *Vibrio*, *Campylobacter*, *Vibrio* and *Listeria* are among the bacteria species that are common in ready to eat foods (Rane, 2011)

In Nigeria, the consumption of roasted plantain and corn cuts across the multi ethnic groups and the various socio-economic classes because of their readily availability and access by any one. Resistance to antibiotics by bacteria is simply the ability of bacteria not to be susceptible to antibiotics previously meant or known for their cure and prevention. The ability of bacteria to resist the effects of antibiotics could either be by acquiring genes that can resist antibiotics or by genetic mutation. O'Neill (2016), has estimated antibiotic resistant bacteria to result to 10 million deaths globally by year 2050. Antimicrobial resistance has become a challenge in achieving universal health coverage and hinder the achievement of sustainable development goals related to health, food security, clean water and sanitation (Interagency Coordination Group on Antimicrobial Resistance, 2019).

The aim of this study was to determine the microbiological analysis of roasted snacks at Ado Main Park and provide information on their susceptibility to antibiotic.



Plate 1: Roasted Plantain

Plate 2: Roasted Corn

Materials and methods

Collection and preparation of samples

Roasted plantains and maize samples purchased from vendor in Ado Ekiti main Motor Park, were collected with sterile forceps into sterile food flask. The samples were transported to the laboratory within 1 hour for microbiological analysis. Ten grams of sample was homogenized with 20 ml of sterile distilled water in a previously sterilized warring blender. From the homogenate, 1 ml of sample was obtained and dilute serially to 10^{-6} .

Sample plating and enumeration of total bacterial count

Using a micropipette, 0.1 ml of each dilution was obtained and spread plated onto Plate Count Agar (PCA) for determination of total bacterial count, macconkey agar for total coliform count and Nutrient agar for general bacteria isolation, The plates were kept in an incubator at 37 °C for 24 h. Average number of colonies in particular dilution was multiplied by the dilution factor to obtain the total viable count. Results of the total bacterial count was expressed as number of colony forming units per gram of sample (CFU/G)

Colony characterization and identification

Colony characteristics such as shape, size, surface texture, edge and elevation and colour developed on nutrient after 24 h of incubation at 37 °C were recorded. The isolated were purified by streaking on freshly prepared nutrient agar. Physiological, morphological and biochemical test were performed on pure isolates according to the criteria of Holt *et al.* (1994).

Antibiotics sensitivity test

Antibiotics sensitivity test was performed by disc-diffusion method. Mueller Hinton agar was prepared and 1 ml broth culture containing about 10^7 cells of test isolate was streaked on the surface of the medium with sterile swabs and left for about 2 h. Antimicrobial discs were placed individually using sterile forceps and then gently press down onto the agar. The plates were inverted and incubated at 37 °C for overnight. After incubation, diameter of inhibition zone excluding diameter of disc was measured in centimetre with a ruler which was then converted to millimetre and recorded as degree of sensitivity.

Statistical analysis

Using the criterion of Ogbeibu (2005), all results in triplicate data were expressed as mean \pm standard deviation. The obtained values were statistically analyzed by analysis of variance (ANOVA) at 50% significance level and least significant difference (LSD) to compare means

Results

Twelve samples each of roasted plantain and corn were obtained for microbiological analysis from which some bacteria species were isolated and characterized to species level. In this study, total bacterial counts (TBC) in roasted corn and plantain sold by different vendors in the main motor park in Ado-Ekiti shows that roasted plantain snack harboured higher bacterial counts than the roasted corn. Highest total bacterial count recorded from roasted plantains was 2.73×10^5 CFU/g and least count of 67×10^3 CFU/g. Total coliform count (TCC) in the roasted plantains snack was between 33×10^3 - 10×10^3 CFU/g. However, two samples out of the twelve sampled were void of coliform contamination. Bacterial load in the roasted maize ranged from

2.14×10^3 to 65×10^3 CFU/g and coliform counts that ranged from 29×10^3 - 2×10^3 was recorded. However, five out of the twelve maize samples were not contaminated with coliform (Table 1). In the current study, seven genera of bacteria species namely: *Shigella dysenteriae*, *Streptococcus faecalis*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella aerogens* and *Staphylococcus aureus* were identified from the surveyed roasted snacks (Table 2).

Percentage occurrence of *B. Cereus* on roasted maize was 28(13.15%) and 38(17.84%) on roasted plantain, rating a total highest occurrence of 66(30.99%). Total percentage occurrence of *E. Coli* in the roasted snacks was 34(15.96%) with maize and plantains having 17(7.98%) each. Others are *K. Aerogenes* with total percentage occurrence of 14(6.53%), *P. Aeruginosa* with 19(8.82%), *S. Faecalis* with 36(16.90%) and *S. Dysenteriae* with 35(16.43%). Meanwhile, *S. Aureus* had the least total percentage occurrence of 9(4.23%), where maize had 4(1.88%) and plantains with 5(2.35%) (Table 3).

Among the isolated bacteria species, *P. Aeruginosa* was the most inhibited as highest inhibition of 18 mm with Cefuroxime was reported. Inhibition of this bacterium was between halos of 18 mm – 3 mm which could not be compared with any other isolate in this study except *S. Aureus*, which was inhibited with the employed antibiotics exempting only nitrofurantoin. Inhibition of *S. Aureus* with the antibiotics ranged from 13 to 7 mm, with cefuroxime acting most on the bacterium followed by ofloxacin with 12 mm halo and least with ceftriaxone which exhibited a halo of 7 mm (Table 4).

Discussion

Roasted plantains harboured more bacterial population than the roasted maize. More importantly, the roasted plantains whose bacterial load were highly populated are the over ripe types. This could be as result of high sugar content and soft nature of the plantains. During postharvest, most of these plantains incurred injuries which will make easy the entrance of microorganisms in the field and during transportation. The less bacterial load encountered in the roasted maize could be as result of the kernels with secured protection by several layers of husk.

Due to the heat process to arrive at roasted plantains and maize, the possibility of the isolated bacteria species from the snacks could either be their high population in the samples, the snacks not well processed, contamination from environment, flies or intended buyers mishandling during pricing. The vendors and producers lack of knowledge of good hygiene practice could also contribute immensely to the contamination of the products. Proper packaging and storage are however not in place thus the products are on continuous exposure to environment till they are bought by customers. Handlers have been implicated as vehicles that spreads pathogenic and indicators bacteria directly or indirectly from the hands to food surfaces (Antonio *et al.*, 2016).

From the perspective of the isolated bacteria species, health hazard is associated to the snacks if not subjected to heat or reheat before sale. The isolated bacteria species in this study are all pathogenic and comparing with the report by Barro *et al.* (2007), the identified bacteria species are of potential health risk. The bacteria identified in this study are in agreement with the report of Ike *et al.* (2015) who has also identified same bacteria species from ready to eat foods. *S. Aureus* isolation from the roasted snacks, could be associated to the dry nature of the products, as it has been reported that this bacterium is a microbial indicator which associates most likely with foods having low water activity (*aw*) (Wallin-Carlquist *et al.*, 2010) or cross contamination from environment and mishandling by intended buyers, vendors or producers. Isolation of *E. Coli*, *S. Dysenteriae* and *S. Faecalis* from the samples is an indication of faecal pollution which might emanate from human hands or flies that perches on the snacks during display for customer's attention. Flies being a habitual feeder have every tendencies to get the snacks contaminated because of unhygienic nature of the producers and vendors. Some of the bacteria species like *E. Coli* and *Klebsiella* identified in this

study have been implicated of producing either endotoxins, heat resistant exotoxins or shigatoxins which have led to high morbidity and mortality rate in man (Valero *et al.*, 2010). The mean bacterial counts in the study was out of range of recommended standard for ready-to-eat foods. According to the report of Jorgensen *et al.* (2017), ready to eat foods microbial load must not exceed $<10^5$ CFU/g. Therefore, this is a pointer to educating producers and vendors about proper packing and storage of ready to eat foods to minimize contamination from environment, insects and intending buyers. Ready to eat foods outside fermented foods that are contaminated with aerobic mesophilic microorganisms irrespective of their nonpathogenic nature, are considered not fit for human consumption (Antonio et al 2016). In Nigeria where there is no policy or regulation on ready to eat foods, this will be most appropriate to control infections from ready to eat foods. However, the recommended microbial load concentration for ready to eat foods should be less than 5.0 log cfu/g, but toxin producing microorganisms like Salmonella, Klebsiella and *E. Coli* species might contradict this statement hence infections of toxin producing bacterial really do not operate on number bases but the amount of toxins produced. Kim *et al.* (2013) have reported similar bacteria species in ready to eat foods examined in Ado. Consumption of food contaminated with pathogens do in most cases cause direct infection like diarrhoea, typhoid fever and several other illnesses depending on the causative agent. Regarding the nature of the isolates response to the employed antibiotics, these bacteria species might be the resistant strains and may transfer their gene encoding resistance to the normal flora of the gut of consumers which might make them highly virulent pathogens that their successful treatment could be difficult to achieve.

The higher total bacteria percentage occurrence in the roasted plantains than the maize is a true reflection of the higher bacteria load evaluated in the respective samples. *Bacillus cereus* being the most occurred bacteria could be traced to its spore forming nature which may give it the protection to survive in a condition thus its more abundance in the environment than the other isolates

Despite *P. Aeruginosa* was the most inhibited bacterium by halo of 18 mm with Cefuroxime in this study, inhibition with nalidizic acid, ofloxacin, tetracyclin and nitrofurantoin were no-significant while it showed no activity with ampicillin, ceftriaxone and penicillin. Likewise, every other identified bacteria in this study suggested either no activity or non-significant inhibition with the employed antibiotics. This signals that the isolates could be resistant strains.

Similar antibiotic sensitivity pattern of 20 – 100% for *S. Aureus*, *E. Coli*, *P. Aeruginosa*, *K. Aerogenes* and *B. Cereus* was reported by Akinyemi *et al.* (2013).

Resistance of microorganisms to antibiotics have been reported to associate with coexistence of resistance genes, plasmids integrons and transposons (Thong and Modarressi, 2011).

Conclusions: Based on the bacteria species identified in this, it could be concluded that the producers or vendors need awareness about microbial contamination and good hygiene practice. This is of paramount important because of the ways the snacks are displayed for customer’s attention, inadequate storage condition and the location of production which is usually roadsides. The traffic movement in these locations are usually very high to promote high microbial contamination of ready to eat foods.

Table 1: Total bacterial counts (TBC) and total coliform counts (TCC) from the vended roasted snacks

Samples	TBC(CFU/g)	TCC (CFU/g)	TFC (SPORE/g)
RM1	$1.29 \times 10^5 \pm 0.1$	$29 \times 10^3 \pm 0.3$	-
RM2	$1.76 \times 10^5 \pm 0.2$	-	$11 \times 10^2 \pm 0.3$
RM3	$1.34 \times 10^5 \pm 0.2$	-	-
RM4	$96 \times 10^3 \pm 0.3$	$16 \times 10^3 \pm 0.2$	-
RM5	$1.37 \times 10^5 \pm 0.2$	$2 \times 10^3 \pm 0.3$	-

RM6	$87 \times 10^3 \pm 0.3$	$17 \times 10^3 \pm 0.3$	-
RM7	$2.70 \times 10^5 \pm 0.1$	-	-
RM8	$1.54 \times 10^5 \pm 0.2$	$10 \times 10^3 \pm 0.2$	-
RM9	$2.14 \times 10^5 \pm 0.2$	$25 \times 10^3 \pm 0.3$	$9 \times 10^2 \pm 0.1$
RM10	$75 \times 10^3 \pm 0.2$	-	-
RM11	$1.26 \times 10^3 \pm 0.2$	$5 \times 10^3 \pm 0.3$	$15 \times 10^2 \pm 0.2$
RM12	$65 \times 10^3 \pm 0.3$	-	-
RP1	$67 \times 10^3 \pm 0.2$	$25 \times 10^3 \pm 0.2$	$10 \times 10^2 \pm 0.1$
RP2	$1.87 \times 10^5 \pm 0.2$	-	$18 \times 10^2 \pm 0.2$
RP3	$2.30 \times 10^5 \pm 0.1$	$10 \times 10^3 \pm 0.2$	$23 \times 10^2 \pm 0.3$
RP4	$98 \times 10^3 \pm 0.2$	$33 \times 10^3 \pm 0.3$	$7 \times 10^2 \pm 0.1$
RP5	$2.21 \times 10^5 \pm 0.2$	$26 \times 10^3 \pm 0.2$	$32 \times 10^2 \pm 0.2$
RP6	$2.13 \times 10^5 \pm 0.1$	$13 \times 10^3 \pm 0.3$	$16 \times 10^2 \pm 0.2$
RP7	$1.79 \times 10^5 \pm 0.2$	$15 \times 10^3 \pm 0.2$	-
RP8	$2.65 \times 10^5 \pm 0.1$	-	$11 \times 10^2 \pm 0.2$
RP9	$2.15 \times 10^5 \pm 0.2$	$30 \times 10^3 \pm 0.2$	$25 \times 10^2 \pm 0.3$
RP10	$2.73 \times 10^5 \pm 0.1$	$23 \times 10^3 \pm 0.2$	$13 \times 10^2 \pm 0.1$
RP11	$2.36 \times 10^5 \pm 0.2$	$12 \times 10^3 \pm 0.3$	$8 \times 10^2 \pm 0.2$
RP12	$1.14 \times 10^5 \pm 0.2$	$13 \times 10^3 \pm 0.2$	-

Legend: TBC = total bacterial counts, TCC = total coliform counts, TFC = total fungal counts, RM = roasted maize, RP = roasted plantain.

Table 2: **Biochemical test result of bacteria isolates from the vended roasted snacks**

Gram rxn	Catalase	Coagulase	Motility	Indole	Citrate	Oxidase	Urease	Colour	Suspected bacteria
+	+	-	+	-	+	+	-	White	<i>B. Cereus</i>
-	+	-	+	+	-	-	-	Glistering	<i>E. Coli</i>
-	+	-	+	-	-	-	-	Grey	<i>S. Dysenteriae</i>
-	+	-	-	-	+	-	+	Glistering	<i>K. Aerogens</i>
+	+	+	-	-	-	-	-	Golden yellow	<i>S. Aureus</i>
+	-	-	-	-	-	-	-	Cream	<i>S. Faecalis</i>
-	+	-	+	+	-	+	-	Green	<i>P. Aeruginosa</i>

Legend: RC = Roasted corn, RP = Roasted plantain

Table 3: **Percentage distribution of bacteria isolated from the roasted vended snacks**

Isolates	Roasted maize	Roasted Plantains	Total (%)
<i>B. Cereus</i>	28(13.15)	38(17.84)	66(30.99)
<i>E. Coli</i>	17(7.98)	17(7.98)	34(15.96)
<i>S. Aureus</i>	4(1.88)	5(2.35)	9(4.23)

<i>K. Aerogens</i>	11(5.16)	3(1.41)	14(6.57)
<i>P. Aeruginosa</i>	10(4.69)	9(4.23)	19(8.92)
<i>S. Faecalis</i>	13(6.10)	23(10.80)	36(16.90)
<i>S. Dysenteriae</i>	15(7.04)	20(9.39)	35(16.43)
TOTAL	98(46.01)	115(53.99)	213(100)

Table 4: Antibiotics sensitivity (mm) pattern of bacteria isolated from the vended roasted snacks

ISOLATE	AMC 30 mg	NAX 30 mg	OFL 10 mg	CRO 30 mg	PCL 10 mg	TET 30 mg	CXM 30 mg	FRT 200 mg
<i>B. Cereus</i>	11	-	3	-	2	6	7	5
<i>E. Coli</i>	6	3	-	6	6	5	4	4
<i>Klebsiella aerogens</i>	3	2	1	2	2	1	2	2
<i>S. Aureus</i>	8	10	12	7	8	10	13	-
<i>P. Aeruginosa</i>	-	4	3	-	-	5	18	10
<i>S. Faecalis</i>	10	-	8	5	-	-	6	-
<i>S. Dysenteriae</i>	12	9	16	12	9	17	3	13

Legend: AMC = Ampicillin, NAX = Nalidixic acid, OFL = Ofloxacin, CRO = Ceftriaxone, PCL = Penicillin, TET = Tetracycline, CXC = Cefuroxime, FRT = Nitrofurantoin

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