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Analysis of airborne bacteria in environmental exposed tiger nuts (*Cyperus esculentus*) sold by street vendors in Abakaliki, Nigeria

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Abstract

Tiger nuts (*Cyperus esculentus*) are tuber crops that are consumed as snack, for their medical and nutritional values. Thus, if exposed to microbial contamination, can impose public health treat. This present study was aimed at analysis of airborne bacteria in environmental exposed tiger nuts (*Cyperus esculentus*) sold by street vendors in Abakaliki, Nigeria. Exactly, six (6) different yellow tiger nut distributed at 10gram each, six (6) different exposed air contaminated Petri dishes samples by passive sampling method were collected randomly from three different traffic points and assessed using standard microbiological techniques. The mean aerobic bacteria coliform counts (CFU) ranged from $1.1\times10^3\pm0.30$ to $3.4\times10^4\pm0.10$ at Abakpa junction, $1.5\times10^3\pm0.10$ to $3.8\times10^4\pm0.16$ at Afikpo road and $2.0\times10^3\pm0.17$ to $3.9\times10^4\pm0.20$ at Vanco junction. Six different bacteria species were detected, namely *Staphylococcus aureus* 11 (25.0%), *Escherichia coli* 10 (22.7%), *Salmonella* species 7 (15.9%), *Shigella* species 5 (11.4%), *Enterobacter* species 5 (11.4%) and *Pseudomonas* species 7 (15.9%). Some of the isolates were resistant to cephalosporin which is one of the major classes of antibiotics commonly available in pharmaceutical stores. Majority of the bacteria isolates were 100% susceptible to gentamicin, chloramphenicol, ciprofloxacin, and imipenem. Multidrug resistances were reported on 9 (20.5%) aerobic bacteria out of 44 isolates studied. The microbial count in this study is above acceptable threshold and presence of multidrug resistances bacteria in tiger nut is a serious public health concern. Regular monitoring of food items by environmental health officers is ideal to avoid outbreak of epidemic as a result of contaminated tiger nuts/food.

1. Introduction

Tiger nut (*Cyperus esculentus*) has been seen as an important tuber crop since ancient time and it is mostly consumed by certain tribes in Africa, although underutilized in agriculture [1]. It was introduced in Europe during the Middle Ages by the Arabs after their expansion across the north of Africa. Today, tiger nuts are cultivated in Northern part of Nigeria, Niger, Mali, Senegal, Ghana, and Togo where they are used in fresh or dry form as snack to argument food nutrient [2]. In Nigeria, tiger nuts are commonly called "Ayaya" in Hausa, "Ofio" in Yoruba, and "Akiausa" in Igbo and are widely grown extensively in the Northern part of the Nigeria; with three different varieties (black, brown and yellow) are cultivated and only two types, yellow and brown, are commonly available in the local market [3]. There has been a significant increase in the consumption of tiger nuts and its products in Nigeria in the last few years and tiger nut is gaining popularity because of its sweetness [4]. Tiger nut tubers has been recognized as a reliable food source in times of food scarcity since tiger nut tubers are readily available

in the market almost throughout the year and it is usually processed into different edible products [5]. The tiger nut (*Cyperus esculentus*) tubers are commonly consumed in raw form, such as dry, fresh and can be mixed with groundnut or other nuts and fruits that are nutritious to the body to produce non-alcoholic dairy-like beverage [6]. The energy value of tiger nut tuber ranges within 400–413.8 kcal/100 g [7]. The main components of tiger nut are carbohydrates, which represent 43.3 g/100g and starch content, 29.9% in wet matter [8].

Tiger nut (*Cyperus esculentus*) has been considered as one of the best important nutritional crop recommended for augmenting diets because of its rich iron and calcium contents that is needed for body growth and development, since a substantial intake has reduced reported cases of various health related disease conditions such as diabetes, obesity, cancer and cardiovascular disease [9]. The consumption of tiger nut (*Cyperus esculentus*) tuber crop has also been discovered to bring about improvement of human reproductive system to its maturity [10]. In folk medicine practice, tiger nut tuber crops were used as a colon evacuator [11]. Consumption of tiger nut tubers contributes in the treatment of boil, common cold and poliomyelitis [12]. Its use as a stimulant and sedative has been reported. The consumption of tiger nut tubers increases digestion process. Treatment of diarrhoea and intestinal inflammation (colitis) can be achieved with the aid of consumption of tiger nut tubers crops [13].

Despite the nutritional quality and health benefit of tiger nut (*Cyperus esculentus*) tuber crop, increased consumption coupled with the associated risk of disease to consumers is a matter of great concern because of the health practice of the harvester, consumers and vendors [14]. However, several other factors predispose these products to contamination such as environmental condition, since it is difficult for one to attest to the hygiene practices of the vendors and the tiger nut are even exposed to the environment without proper covering to protect it from dust and other environmental contaminant [15]. Microbial contamination of tiger nut or food easily occurs and often undetected before the nuts/food are consumed, this has led to food intoxication among the consumers and such organisms are *Salmonella* species, *Bacillus cereus, Clostridium* species, and *Staphylococcus* species *Acinetobacter* species, *Enterobacter* species, *Corynebacterium* species, *Neisseria* species, and *Aeromonas* species [16, 17]. Improper handled tiger nut serves as a good source of food-borne illness when contaminated with pathogenic microorganisms such as *Escherichia coli, Staphylococcus aureus, Streptococcus faecalis*, during harvesting, handling, packaging, storage, transporting, buying and display in street markets [12, 18]. The present study therefore aimed at analysis of airborne bacteria in environmental exposed tiger nuts (*Cyperus esculentus*) sold by street vendors in Abakaliki, Nigeria.

2. Methodology

2.1 Sourcing and preparation of samples

The dry and fresh yellow tiger nuts were purchased from three major different traffic locations (Afikpo road, Abakpa and Vanco junction) randomly in November, 2021 at Abakaliki Ebonyi State, Nigeria. Exactly, six (6) different yellow tiger nut distributed at 10gram each and six (6) different exposed air contaminated Petri dishes samples were collected. The samples were aseptically packaged in sterile ziploc bags, ice packed box and were taken to laboratory within 1hr for bacteriological assessment.

2.2 Experiments

Passive sampling method was used to determine the Index of aerobic bacteria air contamination (IABAC) [19]. This index agrees with the number of CFU counted on a Petri dish with a diameter of 90 mm. In passive monitoring, settle plates (Petri dishes containing nutrient agar) were placed 30

meters away from the settlement of the vendors. The Petri dishes were opened and exposed to the air for specified periods of time (30 minutes) to trap aerobic bacteria in air contamination that may be present in the environment, as they may settle out of the ambient air, and onto the media surface of the Petri Dish. These plates were then taken to Applied Microbiology Laboratory and incubated, analyzed for presence of aerobic bacteria and CFU were interpreted [20].

2.3 Sample processing and analysis

The yellow tiger nuts and Petri dishes containing trapped contaminated air (aerobic bacteria) from different vendors at each location were matched together and assigned the name of the site. This gives exactly three groups. Each group of the yellow tiger nuts was divided into two sub-groups at 10 g each for processing. The tiger nuts were soaked in 15 ml of sterile normal saline, in sterile bottles for 30mintues. After the 30mintues the tiger nuts were washed by shaking the bottles vigorously. The saline was then transferred into a sterile beaker. The process was repeated for three additional washes and all the washes pooled. Aliquots of the pooled saline washes were dispensed into centrifuge tubes and centrifuged at 5000 rpm for 5 minutes. The supernatant was decanted and the sediments or aliquot were re-suspended in 1 ml of saline for bacteriological examination. The processes were repeated for each group of nuts collected. Ten-fold serial dilution was carried out by transferring 1 ml of each sample of tiger nut aliquot into a test tube containing 9 ml of peptone water using sterile pipette and mixed to obtain dilution 10^{-1}. One milliliter (1 ml) of dilution (10^{-1}) was then transferred into another test tube (10^{-2}) containing 9 ml of peptone water. Using separate 1 ml pipette, these transfers were repeated until dilution 10^{-4} was achieved [21].

Fresh tiger nut (*Cyperus esculentus*)

Dried tiger nut (*Cyperus esculentus*)

Photo : Tiger nut (*Cyperus esculentus*)

2.4 CFU enumeration, characterization and identification of bacteria

Identification of bacteria was carried out using the following media, MacConkey agar, mannitol salt agar, Salmonella-Shigella agar, Sheep Blood Agar, Chocolate agar prepared according to manufacturers' instructions. The 0.1 ml aliquot of the wash of tiger nut from each group (10^{-3} and 10^{-4} dilutions) was dispensed aseptically into separate Petri dishes and pours plated with the primary media and incubated in duplicate. Also, the same volumes (0.1 ml) of sterile normal saline were used as a control and were also dispensed aseptically into Petri dishes and pour plated with the media. The plates were kept for incubation at 37°C for 24 hrs. The mean colony forming units (CFU) of the bacterial colonies for each sample in duplicate were determined on each plate after incubation using colony

counting chamber and the results were expressed in colony forming units per milliliter (CFU/mL). Bacteria colonies were further sub cultured to obtain pure cultures for further bacteriological identification. Bacteria identification were done by microscopy, gram staining, morphologic examination, oxidation-fermentation tests and other biochemical tests including catalase test, urease test, triple sugar iron test, indole test and citrate utilization test [22, 23].

2.5 Antibiotics susceptibility test (AST)

This test was done on Mueller Hinton agar by disc diffusion method in line with clinical laboratory standard institute (CLSI) guideline. The antibiotics tested included Penicillin (ampicillin 10µg), Cephalosporin (cefotaxime 30µg), Aminoglycoside (gentamicin 10µg), Quinolone (ciprofloxacin 5µg), Phenicol (chloramphenicol 30µg), Carbapenem (imipenem (10µg), Sulfonamide (sulfamethoxazole 25µg) (Oxoid Ltd., Basingstoke, UK). These antibiotics are the most common used antibiotics available in Nigerian pharmacy stores. A 5 ml fresh culture colony of bacteria suspension was prepared to equivalent of 0.5 McFarland standards. A 1 ml of the suspension was transferred onto the agar plate; the surface of the agar was evenly swabbed using sterile cotton swab stick and allowed to dry. Sterile forcep was used to implant the antibiotic discs to the surface of the agar plate and allowed for 30 minutes for the antibiotics to diffuse before incubation. The plates were then incubated at 37°C for 18–24 hours. Diameters of the inhibition zone around the antibiotic discs were measured and interpreted as sensitive or resistant [24].

2.6 ***Statistical analysis***

Statistical analysis was performed using SPSS 16.0 version software package. Data from this research were evaluated using ANOVA. Aerobic bacteria analyses were obtained in duplicate and results gotten were presented in Mean ± Standard error of mean. Results were considered statistically significant where p value is less than ($p < 0.05$).

3. Results and Discussion

Tiger nut (*Cyperus esculentus*) is a tuber crop that grows freely and has long been recognized as one of the nutritional crops to augment diets. Apart from the beneficial aspect of tiger nuts, tiger nut can be a potential vehicle for pathogenic microorganisms that can lead to food intoxication to consumers and as such a serious threat to the health of the general public [16]. This research therefore discovered tiger nuts tuber to harbor heavy load of aerobic bacteria that can cause serious public health threat if not properly checkmated. Proper washing of tiger nuts has been shown to reduce bacterial counts from this study, since washing with water help dislodge microorganisms. Bacterial colony counts on tiger nuts recorded different bacterial loads. The whole samples collected from the vendors and analyzed showed unacceptable levels of coliforms, which are indicators of poor food hygiene. The least in bacteria colony count was recorded with rehydrated, washed tiger nut from Abakpa junction ($1.1\times10^3\pm0.30$) and dried, unwashed tiger nuts from Vanco junction ($3.1\times10^4\pm0.10$) taking the highest lead in bacterial colony counts (CFU) (Table 1). The high microbial load in these tiger nut tubers from Vanco junction could be linked to heavy environmental exposure, when compared with samples from the other location, as a result of its strategic location to heavy traffic, Vaco junction is the collection point of all the vehicles coming in and going out of Abakaliki town, high pollutions around the area and improper handling of tiger nuts such as exposing to dust, direct sunlight, ambient temperature/environment without covering.

3.1: ***Table 1. Aerobic bacteria colony count (CFU)***

Samples	D/unwashed	D/washed	R/unwashed	R/washed	E/E Plates
Abakpa junction	$2.4\times10^4\pm0.30$	$1.8\times10^3\pm0.06$	$1.7\times10^4\pm0.15$	$1.1\times10^3\pm0.30$	$3.4\times10^4\pm0.10$
Afikpo Road	$2.9\times10^4\pm0.20$	$1.9\times10^3\pm0.25$	$2.2\times10^4\pm0.30$	$1.5\times10^3\pm0.10$	$3.8\times10^4\pm0.16$
Vanco junction	$3.1\times10^4\pm0.10$	$2.8\times10^3\pm0.30$	$2.4\times10^4\pm0.09$	$2.0\times10^3\pm0.17$	$3.9\times10^4\pm0.20$

Key:D/washed - dried and unwashed tiger nuts, D/washed - dried and washed tiger nuts, R/unwashed - rehydrated and unwashed tiger nuts, R/washed - rehydrated and washed tiger nuts, E/E Plates - Environmental exposed plates.

The observation of this research is in agreement with the reports of Ike *et al.* (2017), during their study in Aba metropolis. The result of passive sampling method used to determine the Index of aerobic bacteria air contamination (IABAC) [19], reveal that environmental exposed Petri dishes were heavily contaminated with aerobic bacteria and the bacterial colony forming unity (CFU) were $3.4\times10^4\pm0.10$ at Abakpa junction, $3.8\times10^4\pm0.16$ at Afikpo road and $3.9\times10^4\pm0.20$ at Vanco junction (Table 1), thus significant at ($p < 0.05$). This observation is in line with the study that recorded bacterial load of coliform to range from 1.2 x 10^4 to 3.0 x10^4 [21].

3.2: ***Table 2. Bacteria recovered in tiger nuts from different locations in Abakaliki***

Isolates	Abakpa Junction	Afikpo Road	Vanco Junction	Total (%)
Staphylococcus aureus	4	3	4	11 (25.0)
Pseudomonas spp	3	2	2	7 (15.9)
Salmonella spp	2	2	3	7 (15.9)
Shigella spp	1	2	2	5 (11.4)
Escherichia coli	3	3	4	10 (22.7)
*Enterobacter*spp	1	2	2	5 (11.4)
Total	**13(29.6)**	**14(31.8)**	**17(38.6)**	**44 (100)**

In this present investigation six (6) bacterial organisms, which some of them are associated with serious health implications were observed. A total of 44 bacteria were isolated from tiger nut tubers collected from three location studied, 13 (29.6 %) at Abakpa junction, 14 (31.8 %) at Afikpo road and17 (38.6%) at Vanco junction. Thus, isolation of these bacteria which includes *Staphylococcus aureus* 11 (25.0 %), *Escherichia coli* 10 (22.7 %), *Salmonella* spp7 (15.9 %), *Shigella s*pp 5 (11.4 %), *Enterobacter* spp 5 (11.4 %) and *Pseudomonas* species 7 (15.9 %) (Table 2) on the tiger nuts tubers in this study is a health concern, since some of them can impose serious health problems. *Staphylococcus* spp and *Salmonella* spp have been implicated in food spoilage, food intoxication and food-borne diseases; hence, they should be of paramount interest when food hygiene is being evaluated. This akin with the statement, the intake of contaminated food can lead to diarrhoeal-associated illnesses with bacteria being one of the major causes [25, 26]. The presence of coliforms such *Escherichia coli* and *Enterobacter* spp in tiger nut tubers could be align with improper health hygiene of exposing the nut to dust and sanitary practices of using bare hands by vendors/ hawkers to measure and sell the tiger nuts to the public, and an indication of faecal contamination as observed by the current research. This finding is supported by the report of study on microbial evaluation of tiger nut (*Cyperus esculentus*) sold in Aba [20]. Another research reported that *Escherichia coli* has been identified and used as an indicative microorganism for faecal contamination and its presence in tiger nuts tuber points directly to poor hygiene and sanitary practices by vendors/ hawkers [27]. Thus, hygiene practice of the vendors and packaging methods of

these tiger nut must be monitored basically time to time. This study also observed that most of the isolates in this study may have been introduced into these nuts through faecal polluted water used in washing utensils like wheel barrow, trays, measuring cups and polyethene bags used for the packaging of the tiger nut and also exposure of these nut to dust, environment, and constant rehydration of this tiger nut without covering it encourages the microbial growth of these pathogens and is in agreement with the report of Daniyan and Ajibo, 2011 [28]. The presence of *Staphylococcus aureus, Pseudomonas* spp*, Salmonella* spp and *Escherichia coli* has also been reported in a study done on pre-cut fruits sold in Ilorin [29].

Presence of *Staphylococcus aureus* on tiger nut is an indication of environmental and human contamination. *Staphylococcus aureus* has been implicated in food-borne disease since staphylococcal toxins are common cause of food poisoning. *Staphylococcus aureus* may be expected to exist, if the tiger nut is not properly handled by humans, unless important steps are taken to affect their destruction [30]. *Escherichia coli* is a primary indicator for microbial quality of food and water and this shows that the nuts are not safe for human consumption. According to the Centre for Disease Control, the main transmission of *Escherichia coli* was through faecal contaminated food and water. The high occurrence may have occurred due to contact of contaminated water with tiger nuts during washing of fruits and inadequate washing of hands by vendors [31] and also some vendors have little or no water to rinse all the nuts. Similar work done in River State, Nigeria and Spain reported high level of contamination of aerobic bacteria in tiger nut which includes *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp, *Enterobacter* spp and *Pseudomonas* species [32] and in Spain [33]. Tiger nut tubers gotten from street vendors/hawkers should be properly decontaminated by washing and surface sterilization before eating or processing it into tiger nut fruit drink in order to maintain the microbial load at low level to avoid human food intoxication that can lead to lose of life.

3.3: ***Table 3****. Antibiotics Susceptibility pattern of phenotype bacteria isolated from tiger nuts*

Antibiotics	***S. aureus*11**	***Pseud*sp- 7**	***Salm* sp- 7**	***Shige* sp-5**	***E. coli*- 10**	***Entero*sp- 5**
(µg)	R(%) S(%)	R(%) S(%)	R(%) S(%)	R(%) S(%)	R(%) S(%)	R(%) S(%)
AMP	5(46) 6(54)	3(43) 4(57)	4(57) 3(43)	2(40) 3(60)	4(40) 6(60)	1(20) 4(80)
CTX	4(36) 7(64)	3(43) 4(57)	3(43) 4(57)	1(20) 4(80)	2(20) 8(80)	1(20) 4(80)
GEN	0(0) 11(82)	1(14) 6(86)	0(0)7(100)	0(0) 5(100)	0(0)10(100)	0(0) 5(100)
CIP	1(9) 10(91)	0(0) 7(100)	0(0) 7(100)	0(0) 5(100)	0(0)10(100)	0(0) 5(100)
CHL	0(0)11(100)	0(0) 7(100)	1(14) 6(86)	0(0) 5(100)	1(10) 9(90)	0(0) 5(100)
IPM	1(9) 10(91)	0(0) 7(100)	1(14) 6(86)	0(0) 5(100)	2(20) 8(80)	0(0) 5(100)
SXT	2(18) 9(82)	1(14) 6(86)	2(29) 5(71)	1(20) 4(80)	2(20) 8(80)	1(20) 4(80)

Key: *S. aureus- Staphylococcus aureus; Pseudo* sp - *Pseudomonas* spp; *Salm* sp - *Salmonella* spp; *Shige* sp - *Shigella* spp; *E. coli-Escherichia coli; Entero* sp - *Enterobacter* spp.

It is worthy to note that majority of the isolates were resistant to cephalosporin (ampicillin and cefotaxime) which is one of the antibiotics commonly available in pharmaceutical stores (Table 3), and is a serious public health concern. Multidrug resistance was confirmed when the isolates were found to be resistant to at list two different classes of antibiotics. Thus, *Staphylococcus aureus* (2), *Escherichia coli* (2)*, Salmonella* spp (2), *Shigella s*pp (1), *Enterobacter* spp (1) and *Pseudomonas* species (1) were found to show multidrug resistant (Table 4).

3.4: *Table 4. Multidrug resistance bacteria isolated in tiger nuts from different location*

Isolates	Abakpa Junction	Afikpo Road	Vanco Junction	Total (%)
Staphylococcus aureus	1/4	0/3	1/4	2/11 (18.2)
Pseudomonas spp	1/3	0/2	0/2	1/7 (14.3)
Salmonella spp	0/2	1/2	1/3	2/7 (28.6)
Shigella spp	0/1	1/2	0/2	1/5 (20.0)
Escherichia coli	0/3	0/3	1/4	2/10 (20.0)
*Enterobacter*spp	0/1	0/2	1/2	1/5 (20.0)
Total	**3/13(23.1)**	**2/14(14.3)**	**4/17(23.5)**	**9/44 (20.5)**

Key: *S. aureus* - *Staphylococcus aureus; Pseudo* spp - *Pseudomonas* spp; *Salm* spp - *Salmonella* spp; *Shige* spp - *Shigella* spp; *E. coli* - *Escherichia coli; Entero* spp - *Enterobacter* spp.

The report of this current research on antibiotics resistance akins with the findings of other researchers who worked on multidrug resistant pathogens [34, 35]. *Staphylococcus aureus* were 100% susceptible to gentamicin, chloramphenicol; *Escherichia coli, Salmonella* spp, *Shigella s*pp, *Enterobacter* spp were 100% susceptible to gentamicin, ciprofloxacin, and *Pseudomonas* species were also susceptible to gentamicin, ciprofloxacin, chloramphenicol and imipenem (Table 3). This observation is in line with the report on bacteria isolates from tiger nut in Ghana [21]. The discovery of this current study also agreed with the finding of other researchers who worked on bacteriological and nutritional quality of tiger nut consumed by students of Nasarawa State University, Keffi Nigeria [36]. This observation proved that clinicians and medical officers can still have hope for number of antibiotics choices to select from, during treatment of bacteria diseases acquired as a result of bacteria contaminated tiger nuts consumed by individual from street vendors in Nigeria. This should not stop awareness of the public health implication of bacteria contaminated tiger nut sold in Nigeria by environmental health officers and other government health agencies.

Conclusion

This study has revealed the presence of coliform bacteria in exposed tiger nut (*Cyperus esculentus*) and air trapped from environment to be above acceptable standard, thus was statistically significant at ($p < 0.05$). These coliform bacteria can cause food-borne intoxication that may lead to epidemic in human if not checkmated. This is the first study in Abakaliki Nigeria that was able to isolate multidrug resistant bacteria in tiger nuts and air trapped from environment, although at low level. Multidrug resistance isolates reported include *Staphylococcus aureus* (2), *Escherichia coli* (2), *Salmonella* spp (2), *Shigella s*pp (1), *Enterobacter* spp (1) and *Pseudomonas* species (1). Practice of eating tiger nut improperly wash or unwashed at the point of buying is discouraged, since this practice can inoculate loads of pathogenic bacteria that can cause stomach cremping, diarrhea and possibly lead to death. There is serious need for quality of food items sold in the market and street by vendors to be monitored regularly by environmental health officers to avoid outbreak of epidemic as a result of contaminated food. We therefore, suggest molecular studies on bacteria isolates from tiger nuts to understand the possible antibiotics resistance genes and virulence genes their harbor. We also recommend further studies on isolates from tiger nut, human and environment to understand the clonal relationship, diversity of this multidrug resistant isolates and their role in one health.

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