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BACTERIOLOGICAL ASSESSMENT OF FAST FOODS SOLD AT GIDAN-KWANU AND BOSSO CAMPUSES OF FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA

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ABSTRACT

The bacteriological quality of snacks (meatpie and eggroll) collected from different vendors at two different sale points each at Gidan-Kwanu and Bosso campuses of Federal University of Technology, Minna was carried out in order to ascertain their safety. A total of fourty (40) snacks were screened using standard pour plate method while gram staining and biochemical test were carried out for the identification of various isolates. The samples had varying degree of bacterial contamination ranging from 2.0 x 10^2 - 1.4 x 10^3 cfu/g. The bacteria isolates found include *Bacillus subtilis* (34.12%) in meatpie and (22.45%) in eggroll; *Staphylococcus aureus* (18.82%) in meatpie and (22.45%) in eggroll; *Klebsiella* (22.35%) in meatpie and (22.45%) in eggroll; (4.71%), *Escherichia coli* (20.00%) in meatpie and (26.53%) in eggroll; *Pseudomonas aeruginosa* in meatpie and (4.08%) in eggroll and *Proteus* (2.04%) in eggroll and no growth of *proteus* was recorded in meatpie. The high bacteria count and diversity of bacteria isolated from the food samples screened is of public health concern. The study underscores the need for intervention from bodies charged with the responsibility of maintaining public health to prevent potential outbreak of disease among consumers of these food products.

Keywords: Fast foods, bacterial count, isolation and characterization

INTRODUCTION

Fast foods can be described as foods ready for immediate consumption at the point of sale. Fast foods could be raw or cooked, hot or chilled and can be consumed without further heat treatment (**Tsang, 2002**). Different terms have been used to describe such ready to eat foods. These include convenient, ready, instant and fast foods. Examples of such ready to eat foods include pastries, meat pie, sausage, rolls, burger, *moi-moi*, salad, fried meat, chicken, milk and milk products among others (**Anonymous, 2019a**). A general observation of our society shows a social pattern characterized by increased mobility, large numbers of itinerary workers and less family or home centered activities. This situation however has resulted in more ready to eat foods taken outside home.

According to **Doyle and Evans (1999)**, food borne diseases are diseases resulting from ingestion of bacteria, toxins and cells produced by microorganisms present in food. Data on issues of food borne diseases are well documented worldwide. Food borne illness is a major international health problem with consequent economic implications **(Duff *et al*., 2003)**. In the United States, these pathogens *Escherichia coli* (0157:H7), *Listeria monocytogenes, Campylobacter jejuni, Clostridium perfringes, Salmonella spp., Toxoplasma gondii and Staphylococcus aureus* were reported to be associated with animal products. These pathogens account for approximately 3.3 -12.3 million cases of food borne illnesses and 3900 deaths annually **(Buzby and Roberts, 1997; Anonymous, 2019b)**. Outbreaks of food borne diseases are caused by foods that are contaminated intrinsically or that become contaminated during harvesting, processing or preparation **(Torok *et al*., 1997)**. Another problem associated with consumption of fast food is the adverse effect it has on one's health. It is a fact that fast food is more unhealthy than home-cooked meals, as they contain higher amount of unwanted nutrients like salt, fat and various type of additives (artificial chemicals). Frying destroys most of the essential nutrients from the food and very small amounts of vegetables and fruits are normally present in fast food. Usually when individuals eat too much of fast food, they might become obese and develop disease such diabetes, high blood pressure, stroke, and hearth related symptoms due to high cholesterol from excessive fat.

There is dearth of information on the cases of food borne outbreaks most especially in the developing world, Nigeria included. Outbreaks of food borne diseases are caused by foods that are contaminated intrinsically or that become contaminated during harvesting, processing or preparation **(Torok *et al*., 1997)**.

The problem is further compounded by the in efficiency of regulatory agencies that are constitutionally empowered to enforce good manufacturing practices at all levels of food production and processing. There is therefore need to study the safety and wholesomeness of locally consumed snacks within the two University campuses.

MATERIAL AND METHOD

Sources of samples

Fast foods (eggroll and meatpie) were obtained from four different vendors, two from each the (Bosso and Gidan Kwanu Campuses) of Federal University of Technology Minna, Nigeria.

Collection of samples

A total of 40 samples (20 eggroll and 20 meatpie) were randomly collected from four (4) known regular vendors, two from each campus (Bosso and Gidan-Kwanu) of Federal University of Technology Minna, Nigeria. The samples were collected in a clean polyethylene bag into coolers and transported to Microbiology Departmental Laboratory for further analysis

Plate count

Method described by **AOAC (2000)**. One (1) gram of each sample was weighed into a sterilized test tube containing 9ml sterilized distilled water and serial dilution was carried out for viable count using pour plate method. One (1) ml of each diluted sample was introduced onto petri dish and 15 ml molten nutrient agar was poured onto the diluted sample in the petri dish. The plate was gently swirled to ensure proper mixing of the sample and the media. The plate for nutrient agar was allowed to solidify and incubated at 37^{o}C for 24 h. The same process was carried out for inoculation using Sabouroud dextrose agar at 28^{o}C for 2 - 4 days in an incubation hood. Counting was done using colony counter. The colonies were then recorded as the number of colony forming unit per gram(cfu/g). The bacteria counted include *staphiloccus aureus, proteus, klebsiella, Escherichia coli.*

Characterization and identification of isolates

The pure cultures obtained were used for subsequent examination made on growing colonies and other morphological changes like pigmentation, size, shape, colour, edge and consistency were noted. Other characterization used include gram stain, urease activity, oxidase test, catalase test, coagulate test, indole test and methyl red-Vogues Proskauer **(Cowan and Steel, 2000)**.

Gram staining

A sterile wire loop was used to prepare a smear from the pure culture onto a clean greased free slide containing a drop of distilled water and emulsified to avoid any contamination. The slide was fixed by passing it through the flame three times and allowed to dry and the slides were stained with 0.5% crystal violet (which is the primary stain) for 60 sec., then washed with tap water. Lugol's iodine solution was used to flood the slide for 30 sec. and then washed off with distilled water. Ninety five percent (95%) alcohol (ethanol) was used to decolorize the slide and rinsed with distilled water. The slides were flooded with safranin (which is the secondary stain) for 60 sec. The slides were drained and allowed to dry and were examined under the microscope using the oil immersion objective(x100) **(Tortora *et al*., 2003)**.

Biochemical Test

Catalase Test

This test was carried out to differentiate those bacteria that produce the enzyme catalase such as *staphylococci* from non-catalase producing bacteria such as *streptococci.* A smear of the bacterium was made on the slide using sterilized wire loop. Two (2) drops of 3% hydrogen peroxide was added on the suspension slide. The production of gas bubbles indicated a positive reaction **(Cowan and Steel, 2000)**.

Coagulase Test

This test was used to identify *Staphylococcus aureus* which produces the enzyme coagulase. It is the same procedure with that of catalase test except that human plasma was used in place of hydrogen peroxide. A small portion of the culture was emulsified on a clean slide with the aid of a wire loop. Three drops of undiluted human plasma was added to it and observed for clumping. Coagulation indicated positive result while negative result show no clumping **(Cowan and Steel, 2000)**.

Citrate Utilization Test

This test was carried out by preparing a citrate agar on a petri-dish, making a streak on the isolates with sterile wire loop and incubated at 37ºC for 48 h. A bright blue colour in the medium indicates a positive result **(Cowan and Steel, 2000)**.

Oxidase Test

A piece of filter paper was placed in a clean petri dish and 2-3 drops of freshly prepared 1% oxidase reagent (Tetramethyl-p-phenylene diamine dihydrochloride) was added. Using a glass rod, the test organism was removed and smeared on the filter paper. The development of purple/blue colour within 30 sec. was read as a positive result **(Cowan and Steel, 2000)**.

Urease Test

This was carried out to determine the ability of the isolated organism to produce the enzyme urease for the decomposition of urea. Urea agar slant was inoculated with the different isolates, leaving one slant un-inoculated to act as the control. The slant were incubated at37ºC for 48 h. For positive urease culture, the colour of the medium changes from dark brown to red or purple while no colour change confirms urease negative culture **(Cowan and Steel, 2000)**.

Indole Test

Sterile nutrient broth in a test tube was inoculated aseptically with a loop-full of the isolates and incubated at 37ºC for 48 h. Kovac's reagent (0.5 ml) was added to the 48 h. old broth culture, and then shaken and examined after one min. A red colour in the layer indicates indole production **(Cowan and Steel, 2000)**.

Methyl red-Vogues Proskauer

One (1ml) of the isolate broth was inoculated into 5ml of methyl red-Vogues Proskauer broth and incubated for 24 h. at 37ºC.After the period of incubation, 1ml of the broth was then transferred to a small serological tube and 2 drops of methyl red was then added. Methyl red colour indicates a positive result while a negative test was observed by a yellow colour. To the rest of the broth in the original test tube, 5 drops of 40% potassium hydroxide (KOH) was added and followed by 15 drops of 5% naptha in ethanol. A positive Vogues Proskauer test developed a red colour within 1 h. while a negative test indicates no colour change **(Cowan and Steel, 2000)**.

RESULT

The viable microbial count (Table 1) from the two campuses revealed that Gidan Kwanu has the highest bacteria count in both meatpie and eggroll. The result shows that meatpie snack has a coliform count of $5.0x10^2$ - $1.4x10^3$ while eggroll had 3.0×10^2 - 8.0×10^3 for Gidan Kwanu campus. The count for Bosso campus which was less compared to Gidan Kwanu count was 4.0×10^2 - 1.1×10^3in meat pie and 2.0×10^2 - 4.6×10^3in egg roll. The rise in the bacterial count could be attributed to high number of student activity and movement around the selling spots which may include regular opening of the show glass or materials used in housing the snacks or touching the snacks by the buyers

Table 1 Viable plate count of bacteria from snacks (meatpie and eggroll) samples

Location	Sample	Coliform count (cfu/g)	Aerobic count (cfu/g)
Bosso campus	Meatpie	$4.0x10^2$-$1.1x10^3$	$4.0x10^2$-$1.2x10^3$
	Eggroll	$2.0x10^2$-$4.6.0x10^3$	$2.0x10^2$-$4.6x10^3$
Gidan-Kwanu campus	Meatpie	$5.0x10^2$-$1.4x10^3$	$6.0x10^2$-$1.8x10^3$
	Eggroll	$3.0x10^2$-$8.0x10^3$	$3.1x10^2$-$1.8x10^3$

Key: cfu/g=colony forming unit per gram

this could be a contributing factor in the high number of pathogens. The snacks could also be exposed to contamination by the vendor or the seller during sale by direct hand contact.

In this study, it was observed that most of the samples analyzed were contaminated with pathogenic bacteria. This result is similar to the findings of **Orunisi *et al.* (2011)** who reported the presence of pathogenic bacteria in fast foods sold in shops in Ota, Ogun state, Nigeria. The high frequency of bacteria (Tables 2 & 3) and their respective percentage occurrence (Tables 4 & 5) observed in the meatpie as compared to the eggroll might be as a result of the quality of the meat and meat spice added during the production of the snack. The production of these snacks are mostly done in nearly unhygienic condition such as using untreated water, contaminated utensils and coupled

Table 2 Frequency of occurrence of bacteria isolated from meat pie

Organism isolated	Location		
	Bosso campus	Gidan-Kwanu campus	Total
Bacillus subtilis	10	19	29
Staphylococcus aureus	9	7	16
Escherichia coli	11	6	17
Klebsiella spp.	11	8	19
Pseudomonas aeruginosa	2	2	4

Table 3 Frequency of occurrence of bacteria isolated from egg roll

Organism isolated	Location		
	Bosso campus	Gidan Kwanu campus	Total
Bacillus subtilis	8	3	11
Staphylococcus aureus	7	4	11
Escherichia coli	10	3	13
Klebsiella sp	9	4	11
Pseudomonas aeruginosa	0	2	2
Proteus sp	0	1	1

with the fact that producers of the snacks have little or no knowledge of maintaining hygiene or safety to prevent contamination. The results revealed that *Bacillus subtilis* 29 (34.12%) had the highest total frequency of bacteria isolated followed by *Klebsiella spp.* 19 (22.35%), *Escherichia coli* 17 (20.00%), *Staphylococcus aureus* 16 (18.12%) and *Pseudomonas aeruginosa* 4 (4.71%). In Gidan Kwanu campus, *Bacillus subtilis* (19) was found to be high in meat pie while *Pseudomonas aeruginosa* (2) had the least frequency. The highest total frequency of bacterial occurrence in egg roll was found to be *Escherichia coli* 13 (26.53%) while *Proteus spp*. 1 (2.04%) had the least total frequency. Unlike in meat pie, egg roll sold in Bosso campus had high frequency of all the bacteria

isolated except *Pseudomonas aeruginosa* and *Proteus spp*. which were not detected in the egg roll.

Table 4 Percentage of bacteria isolated from meatpie

Organism isolated	Frequency of occurrence	Percentage of occurrence
Bacillus subtilis	29	34.12
Staphylococcus aureus	16	18.12
Escherichia coli	17	20.00
Klebsiella spp.	19	22.35
Pseudomonas aeruginosa	4	4.71
Total	85	100

Table 5 Percentage occurrence of bacteria isolated from eggroll

Organism isolated	Frequency of occurrence	Percentage of occurrence
Bacillus subtilis	11	22.45
Staphylococcus aureus	11	22.45
Escherichia coli	13	26.53
Klebsiella spp.	11	22.45
Proteus spp.	1	2.04
Pseudomonas aeruginosa	2	4.08
Total	49	100

The microorganisms isolated (Table 6) in both meat pie and egg roll snacks *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella spp*. and *Proteus spp*. agree with the study by Musa and **Akande (2002)**. *Bacillus subtilis* having the highest total frequency of occurrence in the two snacks and in both campuses could be as a result of the unique nature of the microorganism in its ability to form endospore which confers it resistance to hash environmental conditions including frying. *Staphylococcus aureus* is a normal flora of the skin of a man and might have been transmitted from the handlers to the product through unhygienic practice. The presence of *E. coli* on the other hand could be traceable to contamination from fecal origin. Although, *E. coli* has been shown to exhibit a high desiccation tolerance, contamination of snacks (meat pie and egg roll) with the pathogen could be prior or during the production process of the snacks. This is in agreement with **Tsang (2002)**. The isolation of *Pseudomonas* and *Klebsiella* species from the snacks (meatpie and egg roll) samples is an indication of possible post production contamination as these organism are less heat tolerance hence, expected to have been destroyed by high temperature during baking. However, gram negative aerobic rod shaped bacteria especially pseudomonas specie has been reported as dominant meat product spoilage organism **(Dainty and Mackey 1992, Borch *et al*., 2009)**. The contamination of these products by *proteus* specie could be due to soil or water contamination during processing of the snacks. It could also be inherent; as *proteus* is a protyolitic bacteria as such it is traceable to meat.

Table 6 Characterization of bacteria isolate from both campuses

GR	CAT	COU	MR	VP	UR	OXI	H_2S	G	L	S	CIT	MSA	IND	Possible organism
Positive rod	+	-	-	+	-	-	-	-	-	+	+	-	-	*Bacillus subtilis*
Positive cocci	+	+	-	-	-	-	-	+	-	+	-	+	-	*Staphylococcus aureus*
Negative Rod	+	-	+	-	-	-	-	+	+	+	+	-	+	*Escherichia coli*
Negative rod	+	-	-	+	-	+	-	+	+	+	+	-	-	*Psuedomonas aeruginosa*
Negative Rod	-	-	-	+	-	-	-	+	+	+	+	-	-	*Klebsiella spp.*
Negative Rod	-	-	-	-	+	-	+	+	-	+	-	-	-	*Proteus spp.*

Key: GR= Grams Reaction; H_2S=Hydrogen sulphide production; CAT=Catalase Test, CIT= Citrate Utilisation Test; COU = Coagulase Test; MSA= Mannitol salt Agar Test; MR= Methyl Red Test; IND= Indole Test; VP=Vogues Proskauer Test; UR=Urease Test, OXI = Oxidase Test; S=Sucrose; G= Glucose; L=Lactose

CONCLUSION

This study shows that snacks sold at Bosso and Gidan Kwanu campuses of Federal University of Technology, Minna were contaminated by pathogenic organisms. The findings of this research work have a serious implication for public health management, since the consumption of snacks (meat pie and egg roll) cut across all ages and gender of the society. Therefore, it is imperative to provide intervention measures to prevent or reduce the rate of food contamination by these pathogenic bacteria.

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