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Evaluation of the Microbial Contamination of Some Snacks: A Case Study of Lagos Mainland, Lagos State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors OPO and KOY designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author BUO managed the analyses of the study. Authors NPU and GCN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study was undertaken to evaluate the microbial contamination of some locally prepared snacks sold by street vendors in Lagos mainland, Lagos State, Nigeria. A total of (20) twenty snack samples- Meat-pie, Sausage roll, Egg roll, Puff puff and Doughnut were aseptically purchased in a sterilized polythene bags from four different locations in Lagos Mainland Local Government Area. The snacks were analyzed by standard microbiological methods using Bergey's manual to determine the colony-forming units per gram, to isolate and determine the number of microbiological population contaminants. Unilag showed the bacteria count on snacks as follows: Meat-pie 1.50 × 10^6 cfu/ g), Sausage 2.20× 10^6 cfu/ g, Doughnut 3.20× 10^5 cfu/ g, Puffpuff 1.58x 10^6 cfμ/ g and Egg roll 9.80x10^5 cfμ/ g. At Yaba - Meat pie had bacteria count of 1.82x10^2cfμ / g, Sausage 7.00x10^1cfμ / g, Doughnut 1.09x 10^2 cfμ /g, Puff puff 3.64x10^4 cfμ/ g and Egg roll

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5.00x10[1] cfμ/g. Snacks from Oyingbo had no growth on Meat pie, Doughnut and Puff puff but had bacteria cou nts on Sausage (1.00x10[2]) and on Egg roll (1.95× 10[5] cfμ/ g).At Abule-Oja- Meat pie had bacteria count of 9.75x10[4] cfμ/ g, Puff puff 1.65x10[5] cfμ/ g and Egg roll 1.95x10[5] cfu/ g.The location with lowest bacteria count was Oyingbo which had no growth on a Meat pie, Doughnut and Puff puff, but had on Sausage 1.00x10[2] cfμ/ g and Egg roll 9.60x10[2] cfμ/ g. There were no coliforms in all the locations. Bacteria percentage range was 10.52% to 36.84%while Fungi percentage range was 0.71% - 37.59%. Four bacteria and seven fungi were identified: *Bacillus cereus, Bacillus substillis, Staphylococcus aureus, Lactobacillus delbruckii* with *Aspergillus niger, Penicillium notatum*, *Trichoderma* spp*., Aspergillus fumigatus, Saccharomyces cerevisiae, Fusarium* spp*. and Aspergillus flavus*. *Bacillus cereus* had the highest prevalence of 36.84%, *Bacillus substillis* 31.58%, *Staphylococcus aureus* 21.05% and *Lactobacillus delbruckii* 10.52%. *Bacillus species* were present at all sampled sites. It is concluded that the quality of these snacks can be improved by following quality control protocol and good manufacturing practices (GMP) in food.

Keywords: Snacks; street-foods; bacteria; fungi; Nigeria.

1. INTRODUCTION

Ready-to-eat food" means food which is in a form that is ready for immediate consumption or reasonably expected to be consumed in that form at the point of sale or which is edible without additional preparation to achieve food safety. Such food may be raw, cooked, hot or chilled, and may be consumed without further heat-treatment, including, without limitation and reheating [1]. Street foods are “ready-to-eat” foods and beverages prepared and sold by vendors and hawkers especially in the streets and other similar public places and their poor microbiological quality has often been verified. [2,3]. Street foods can be described as the food sold by street vendors or beverages ready for consumption, prepared and/or sold in public places without the need for another process or preparation [4].

Snacks are referred to as ready to eat foods which are eaten by all age groups with high popularity amongst school children and youths. According to guidelines for the microbiological examination of ready - to - eat foods, snacks are foods that are consumed in the same state as that in which it is sold and does not include nuts in shell and whole, raw fruits and vegetables that are intended for hulling, peeling or washing by the consumer [5]. Foods in form of snacks are prone to contamination at every stage in the food processing and preparation i.e. the various stages from primary production of food to when it is ready for consumption (often described as ‘farm –to –fork’) [6].The consumption of food that has been contaminated by microorganisms can result in food-borne diseases. These are usually either infectious or toxic, caused by agents that enter the body through ingestion of food [7]. In Nigeria, several foods have been reported to have a high incidence of bacterial contamination [8,9,10].

Food-borne illness is a disease usually either infections or toxins, caused by agents that enter the body through the ingestion of food [11]. The microbial agents that cause food-borne illness may include bacteria such as *Salmonella, Staphylococcus aureus,* diarrheagenic *Escherichia coli* (pathogenic strains) *Bacillus* species, *Clostridium botulinum and Listeria monocytogens*. Also, viruses such as hepatitis A and E, Norovirus, moulds, fungi, and yeast cause illness [12]. Other infections that are contacted through food are amebiasis (*Entamoeba histolytica*), *Blastocystis hominis*, cholera, cryptosporidiosis (crypto), *Cyclospora cayetanensis,* viral gastroenteritis, giardiasis, listeriosis, marine toxins shigellosis, travellers, diarrhoea, trichinosi (trichinellosis), typhoid, *Vibrio parahaemolyticus* and *Vibrio vulnificus* infection [13].

WHO [14] states that the most frequent causes of food-borne illness were diarrhoeal disease agents, particularly norovirus and *Campylobacter* spp. It reported that food-borne diarrhoeal disease agents caused 230,000 (95% UI 160,000–320,000) deaths, particularly non-typhoidal *Salmonella enterica* (NTS, which causes diarrhoea and invasive disease). Also, *Salmonella typhi, Taenia solium*, hepatitis A virus, and aflatoxin can be contacted through food that is not properly prepared or preserved before consumption.

Besides, poisonous chemicals or other harmful substance can cause food borne diseases if they are present in food and some can cause organ

failure [15]. The US CDC estimated that each year roughly 48 million people in the US get sick, 128,000 are hospitalized, and 3,000 die from food borne diseases [16]. The foods implicated in food borne disease outbreaks in the studies conducted in the USA are fish, seafood, liver pate, chicken products, meat and meat products, ice cream, raw milk, rice dishes, pasta and pasta salad, peanuts, flour, cold sandwiches, fruit juices and fresh produce [17].

Nigeria had a history of developed supermarket industry until social and economic changes in the early 1980s diminished the country`s middle class significantly since then most Nigerians shop at traditional open-air markets or purchase their goods from traders and street vendors [18]. Street foods are tasty, ready-to-eat or drink and are sold on the street, in markets, parks or other public places. About 2.5 billion people worldwide eat street food every day, according to a 2007 Food and Agriculture Organization study [19]. Unfortunately, most handlers of street food in the developing countries are ignorant of basic food safety measures, good hygiene, environmental cleanliness and good sanitary conditions [20]. Also, street foods are commonly exposed to various contaminants at different stages of handling [21].

In developing countries such as Nigeria, there are serious concerns about the sanitation of ready– to–eat foods; particularly as potable water is seldom available at preparation venues and also most food handlers lack basic knowledge of proper personal and environmental hygiene [22]. Also, a study showed that street food vendors in Benin City have poor knowledge of food hygiene and safety, their practice of food hygiene and safety fell short of standard requirements [23]. As part of continuous evaluation of street food, this study was conducted to isolate and determine the level of microbial contaminants in snacks sold in some areas of Lagos mainland, Lagos State Nigeria.

2. METHODOLOGY

2.1 Samples Collection

Samples were collected in the month of October and November 2020. Samples were randomly collected from four different locations - Yaba, Oyingbo, Abule-Oja and University of Lagos community at Lagos Mainland Local Government Area. The samples were bought in the early hours of the day and brought in polythene bag aseptically and taken to Microbiology Department laboratory, the University of Lagos for immediate analysis. A total of (20) twenty samples of Meat-pie, Sausage roll, Egg roll, Puff puff and Doughnut were aseptically purchased in a sterilized polyethene bags.

2.2 Snacks Making Procedure to Packaging

a) Meat-pie: The basic ingredients in Meat –pie are meat, pastry and vegetables. The chemical content of meat –pie is water, carbohydrates, minerals. Meat –pie is made by making the piecrust, rolling the piecrust, shape the edges, add the fillings and then bake for about 50 minutes.
b) Sausage: This is made from ground meat, salt and spices. There are different types of sausages with varied percentages of moisture, protein starch and ash. Sausages are made by mixing of grounded meat and the flavourings, rolled and bake in a 350 degrees Fahrenheit preheated oven for 30 to 35 minutes.
c) Egg roll: The chemical component of Egg roll is phosphorous, sulphur, potassium, sodium and chloride. It is made up of shredded cabbage, chopped pork meat and other ingredients fried in hot oil
d) Puff puff are made from yeast dough containing eggs, butter, water, flour, yeast and sugar shaped into balls and deep-fried until golden brown.
e) Doughnut is basically made of flour and eggs creamed with butter and sugar fried in hot oil or baked in an oven.

2.3 Samples Preparation

The samples (Meat-pie, Sausage roll, Egg roll, Puff puff and Doughnut) were blended aseptically with sterile mortar and pestle. Serial dilution was made up to ten-fold dilutions for each prepared samples by weighing 5g of the snacks sample into 45mls of sterile distilled water. Media was sterilised at 121^0C for 15 minutes before inoculation of the sample into the media.

A serial dilution of tenfold was carried out and 0.1ml aliquot was placed out on each sterile agar for 15minutes for bacteria and in room temperature for 3-7 days for fungi. After incubation, the visible colonies were sub-cultured and stored on slants for further studies. These were carried out to obtain discrete colonies for each sample [23,24].

2.4 Plating and Culturing

0.1ml of each sample was taken from 1-10 tenfold dilution and the streaking plate method was used. Nutrient agar media was used for the aerobic viable count, MacConkey agar for isolation of gram-negative microorganism according to Oje, et al. [25] and eosin methyl blue agar for enteric organisms and Potato dextrose agar (PDA) for fungi. The isolation was done over one corner of the plate agar which has sufficiently dried. The wire loop was sterilized over a Bunsen flame, cooled and used to make parallel streak from the main inoculating plate. The plates were inoculated at 37^0C for bacteria in 24 hours and 25^0C for 3 to 7 days for fungi respectively before being read.

2.5 Morphology and Cultural Examination

After incubation, the macroscopic cultural characteristics of the growth such as shape, colour, size, elevation and consistency on various media were observed and recorded. The isolates were purified using the reappeared subculture streak plate technique after which the pure colonies were confirmed by Gram staining. The cultures were subsequently stored in the refrigerator for further identification [26].

2.6 Identification of Isolates

The bacteria isolates were identified conventionally based on standard microbiological methods from Bergey's manual [27]. Cultural characteristics and biochemical tests were made - Gram's staining, catalase, oxidase, coagulase, Indole test, methyl red tests, Voges Proskauer, Urease test, citrate tests, nitrate reduction test and motility. Identification of fungal isolates was based on their macroscopic characteristics.

3. RESULTS

Table 1 shows the total heterotrophic bacteria count on nutrient agar plates. Unilag showed the bacteria count on snacks as follows: Meat-pie 1.50×10^6cfµ), Sausage 2.20×10^6cfµ, Doughnut 3.20×10^5cfµ, Puff puff $1.58\text{x } 10^6$cfµ and Egg roll $9.80\text{x}10^5$ cfµ. At Yaba - Meat pie had bacteria count of $1.82\text{x}10^2$cfµ, Sausage $7.00\text{x}10^1$cfµ, Doughnut $1.09\text{x } 10^2$cfµ, Puff puff $3.64\text{x}10^4$cfµ and Egg roll $5.00\text{x}10^1$.cfµ Snacks from Oyingbo had no growth on Meat pie, Doughnut and Puff puff but had bacteria counts on Sausage ($1.00\text{x}10^{2)}$ and on Egg roll (1.95×10^5cfµ).At Abule-Oja - At Abule-Oja - Meat pie had bacteria count of $9.75\text{x}10^4$cfµ /g, Puff puff $1.65\text{x}10^5$cfµ /g and Egg roll $1.95\text{x}10^{5.}$ There were no bacteria counts in Sausage and Doughnut.

The location with the lowest bacteria count is Oyingbo which had no growth on the Meat pie, Doughnut and Puff puff, but had $1.00\text{x}10^2$ cfµ /g on Sausage and $9.60\text{x}10^2$ cfµ / g on Egg roll.

Table 2 showed the Bacteria isolated from twenty snack samples analyzed based on their location, specific snacks and presumptive organisms gotten from them. Those presumptive organisms are *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis and Lactobacillus delbrueckii.*

The organisms were identified morphologically and through biochemical tests.

Staphylococcus aureus morphologically showed a circular yellow colony on nutrient agar and culture gave golden yellow on Mannitol Salt Agar (MSA). The organism was non spore former, easily emulsify during staining and produced beta haemolysis on blood agar. From biochemical test the organism was DNASE positive, Oxidase negative, Catalase positive, fermentatively arranged in clusters. It was acidic from mannitol, trehalose and sucrose were all positive while, from xylose it was negative.

Bacillus cereus were Gram + rod shape. They were spore former that could be round or oval, central or sub-terminal. They were 10x1micrometer in diameter, motile, catalase positive and oxidase negative. Also, they produced acid from glucose, from xylose they were negative, hydrolysis from starch was positive, anaerobic growth was positive and growth at 60^0 C was also negative.

Table 1. Mean count of the Bacterial (cfu / g) Load obtained from different Snacks sources

	Locations			
Snacks	**Oyingbo**	**UniLag**	**Yaba**	**Abule-Oja**
Meat pie	ND	$1.50\text{x}10^6$	$1.82\text{x}10^2$	$9.75\text{x}10^4$
Sausage	$1.00\text{x}10^2$	$2.20\text{x}10^6$	$7.00\text{x}10^1$	ND
Doughnut	ND	$3.20\text{x}10^5$	$1.09\text{x}10^2$	ND
Puff-puff	ND	$1.58\text{x}10^6$	$3.64\text{x}10^4$	$1.65\text{x}10^5$
Egg roll	$9.60\text{x}10^2$	$9.80\text{x}10^5$	$5.00\text{x}10^1$	$1.95\text{x}10^5$

ND = No growth, Unilag = University of Lagos community

Table 2. Bacteria isolated from twenty snack samples analyzed

Location	Snacks	Presumptive Organisms
Oyingbo	Egg roll	*Bacillus cereus, Staphylococcus aureus*
	Meat pie	*Bacillus subtilis and*
	Doughnut	*Staphylococcus aureus*
	Sausage	Absent
	Puff Puff	Absent
Abule-Oja	Doughnut	*Bacillus subtilis and Bacillus cereus*
	Puff puff	*Bacillus subtilis*
	Meat pie	*Bacillus cereus*
	Egg roll	*Lactobacillus delbrueckii*
	Sausage	*Staphylococcus aureus and Bacillus cereus*
Unilag	Sausage	*Bacillus subtilis*
	Egg roll	Absent
	Meat pie	Absent
	Puff puff	Absent
	Doughnut	Absent
Yaba	Puff Puff	*Bacillus subtilis* and *Bacillus cereus*
	Egg roll	*Bacillus subtilis*
	Sausage	*Lactobacillus delbrueckii and Bacillus cereus*
	Doughnut	*Bacillus cereus*
	Meat pie	Not detected

Table 3. Fungi isolated from twenty snack samples analyzed

Locations	Type of Snacks	Presumptive Organisms
Yaba	Doughnut	*Aspergillus niger, Penicillium notatum* and *Trichoderma* spp
	Egg roll	*Aspergillus fumigatus, Saccharomyces cerevisiae* and
	Puff puff	*Penicillium notatum*
	Meat pie	*Fusarium* spp*., Aspergillus niger and Saccharomyces*
	Sausage	*cerevisiae*
Oyingbo	Doughnut	*Aspergillus niger*
	Egg roll	Absent
	Puff puff	*Aspergillus fumigatus*
	Meat pie	*Aspergillus flavus* and *Fusarium* spp
	Sausage	*Aspergillus fumigatus* and *Penicillium notatum*
Abule -Oja	Doughnut	*Aspergillus niger* and *Saccharomyces cerevisiae*
	Egg roll	*Penicillium notatum* and
	Puff puff	*Saccharomyces cerevisiae*
	Meat pie	*Aspergillus niger* and *Trichoderma* spp
	Sausage	*Trichoderma* spp and *Saccharomyces cerevisiae*
		Trichoderma spp and *Saccharomyces cerevisiae*
Unilag	Doughnut	Absent
	Egg roll	Absent
	Puff puff	Absent
	Meat pie	Absent
	Sausage	*Saccharomyces cerevisae*

Lactobacillus delbruckii were gram positive rods of regular shape and not spore formers, They were motile in nature, nitrate were not reduced, gelatin was not liquefied, the cell was catalase positive and oxidase negative.

Table 3 shows Fungi isolated from twenty snack samples analyzed which are *Aspergillus niger, Penicillium notatum*, *Trichoderma* spp*, Aspergillus fumigatus, Saccharomyces cerevisiae, Fusarium* spp *and Aspergillus flavus.*

Table 4 shows the morphological, microscopy, fungal isolated, frequency and percentage of Fungi isolated from twenty snacks samples analyzed. The *Penicillium notatum* had the highest with37.59%, *Saccharomyces cerevisiae* had 24.11%, *Aspergillus niger* had 16.31%, *Trichoderma* spp. 7.80%, *Aspergillus fumigatus* 7.80%, *Fusarium solani* 5.69% and least of all is *Aspergillus flavus* with 0.71%.

4. DISCUSSION

This work revealed that all the snack samples were contaminated with different bacteria and fungi species. The safety of filled savoury pastry products relies largely on adequate handling and hygiene practices. The total aerobic plate count of the resulting cultures from this study ranged from $5.00x10^1$ cfu / g to $2.20x10^6$ cfu / g, the samples did not have coliforms; fungi percentage range from 0.71-37.59. Different bacteria and fungi were isolated from the samples and identified using morphology, microscopy and biochemical tests with Bergey's manual.

The organisms isolated were *Bacillus cereus*, *Bacillus substillis*, *Staphylococcus aureus*, *Lactobacillus delbruckii, Aspergillus flavus, Aspergillus niger*, *Penicillium notatum*, *Fusarium* spp*, Saccharomyces cerevisiae*, *Trichoderma spp.* and *Aspergillus fumigatus*. The presence of *Staphylococcus aureus* is indicative of human contamination after production. This could be from direct human contacts such as fingers or indirectly through additives or utensils. The result is in agreement with the results of [28]. They reported that 9.4% of ready-to-eat-food meats sampled were contaminated with toxigenic and multidrug resistance CoNS. Also, they emphasized that meat samples from motor parks in Enugu State, Nigeria had the highest staphylococcal contamination (16.7%), while those from the mechanic village had the least (2.4%). Similarly, in Yenagoa Bayelsa State, Nigeria, seventy (70) *Staphylococcus* spp. were isolated, consisting of 65 (92.86%) *S. aureus* and 5 (7.14%) coagulase negative *Staphylococcus* (CoNS) [29]. Also, the systematic review of articles published from 2015 to 2020 showed that *S. aureus* in ready to eat foods has the highest pooled prevalence of 46.3% (95% CI: 24.8, 69.4%) more than *E. coli* and *Salmonella* [30]. The organism is associated with endotoxin characterized by short incubation period (1-8 hours), violent nausea, vomiting and diarrhoea [31,32]. Also, the presence of *staphylococcus aureus* could be traced to the fact that it is abundant in the human body (skin, nails, hair) [33]. *Staphylococcus aureus* is normally found on the skin which can cause urinary tract infection [34]. According to Ayoade [35], the chance of the microorganisms getting into the snacks is high when the vendors and the customers sneeze, blow their noses or blow air into the polythene bags to open them. This result is in agreement with the work of [36,37] among others.

Table 4. Morphology and Microscopy result of fungal isolates

Morphology (%)	Microscopy	Fungal Isolates	Frequency
Colonies are white and cottony	Cells have low floccose and shiny sporadical and chlamydospores are brown and round	*Fusarium solani*	8 (5.67)
Colonies are greenish	Conidial head spore stained with lactophenol	*Aspergillus flavus*	1(0.71)
Colonies are greyish green with central area raised	Vegetative hyphae creeping	*Penicillium notatum*	53(37.59)
Blackish brown to black colonies nature	Vegetative mycelium septate	*Aspergillus niger*	23(16.31)
White mucoid and convex colonies	Oval shaped in the bud	*Saccharomyces cerevisiae*	34(24.11)
White colony growth spread like cobwebs		*Trichoderma spp*	11(7.80)
Colonies are smoky-green colour in nature	Colonies of this fungus produce from conidiophores	*Aspergillus fumigates*	11(7.80)
Total		**141**	**100**

Bacillus cereus isolated had the highest frequency and this may be associated with the production of the toxin, diarrheal and emetic in food which causes food poisoning [38]. Similarly, Nwachukwu et al. [39] reported a high-frequency occurrence 59 (9.5%) of *Bacillus* spp. in fast food sold in different grades of mobile food vendors and canteens in Owerri Metropolis, Nigeria. *Bacillus* spp. can be found in dust, soil and raw food and can survive normal cooking as a heat resistant spore [40].

The presence of *Penicillium* spp., *Aspergillus niger* and *Aspergillus flavus* in the food sample is not surprising as they are dispersed in the form of spores which are abundant in the environment and can be introduced through dust and soil. Mixed culture has a higher potential (37.08%) to inhibit the growth of *Aspergillus flavus* (producer of Aflatoxin) compared to either single culture, *L. rhamnosus NRRL B-442* and *S. cerevisiae* [41]. Modupeade et al. [42] reported that in supermarket or roadside-vend peanuts, *A. fumigatus, A. niger* and *A. flavus* were prevalent (>40% incidence) in Southern Africa. Their presence in these food samples is of a serious public health concern as these fungi have all been implicated with the production of mycotoxin. Mycotoxins are naturally occurring toxins produced by certain moulds (fungi) and can be found in food. The moulds grow on a variety of different crops and foodstuffs including cereals, nuts, spices, dried fruits, apples and coffee beans, often under warm and humid conditions. They can cause a variety of adverse health effects and pose a serious health threat to both humans and livestock. The adverse health effects of mycotoxins range from acute poisoning to long-term effects such as immune deficiency and cancer [43].

Most of the snacks from the University of Lagos community have low microbial population.
It might be as a result of the routine check by personal hygiene officers through the school once every week. Such close monitoring is being done to make sure that the foods served in the institution are of good quality and none contaminated.

5. CONCLUSION

It is concluded that the snacks sold in Lagos mainland Local government of Lagos State are contaminated hence the presence of the isolated bacteria and fungi organisms. This may be as a result of poor food handling, poor personal hygiene, dirty packaging materials or vendors and consumers' poor attitudes towards food safety. Therefore, a comprehensive assessment of the methods of human exposures to these pathogenic microorganisms that thrive on snacks should be taken from time to time. Public awareness about the potential risk in the ingestion of these snacks should be aggressively embarked on in the study areas. The regulatory governmental agencies like National Agency for Food and Drugs Administration and Control (NAFDAC) should be actively involved in making sure that food consumed by the people would not constitute health hazard and risks to the inhabitants/consumers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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