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Microbial quality of street prepared and vended snacks in Umuahia town, South East Nigeria

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ABSTRACT

The study evaluated the microbial characteristics of street prepared and vended snacks as well as their production environment at different locations in Umuahia, Abia State. Twenty-five (25) samples of puff-puffs, buns and fish rolls as well as swaps from production tables and surrounding floors were randomly sampled from five locations within Umuahia town using the stratified random sampling procedure. One sample each of fish roll, buns, puff-puff, swab from tables and swab from floors were further randomly selected and subjected to microbial analysis using standard microbiological methods for the determination of total viable count, fungi count, *Lactobacillus* count, *Staphylococcus,* total coliform count, *Salmonella/Shigella* count, and *Streptococcus* count. Discoveries were that the snacks collected contained different microorganisms ranging from food grade types such as lactic acid bacteria to highly pathogenic types such as *Staphylococcus aureus*. Total viable count ($28.01^{a}\times10^{6}$ - $60.70^{c}\times10^{6}$), fungi count ($15.56^{a}\times10^{6}$ - $37.35^{c}\times10^{6}$), and *Lactobacillus* count ($9.34^{ab}\times10^{6}$ - $12.45^{c}\times10^{6}$) were above acceptable limits; *Staphylococcus* was detected only in puff-puff ($12.45^{a}\times10^{6}$ cfu/g); total coliform count, *Salmonella/Shigella* count and *Streptococcus* count were not detected in all the snacks. Most of the microbial characteristics assessed such as total viable count ($37.45^{a}\times10^{6}$ - $62.25^{a}\times10^{6}$) and *Staphylococcus aureus (*$7.78^{a}\times10^{6}$ - $21.0^{a}\times10^{6}$ cfu/g) were present above acceptable limits both in the floor and service table; only *Lactobacillus and Coliform* were not detected in the floor and service table respectively. The study indicates poor microbial quality of the street-vended food and a highly contaminated production environment posing significant risks of food borne illness, and suggests a need for improved hygiene practices, monitoring and enforcement of food safety regulations.

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1. Introduction

In Nigeria, consumption of street foods has witnessed a phenomenal growth. Over the years, rapid population growth, urbanization, unemployment and poverty, occupational pressures and lifestyle changes have created a poll of mobile and transient population who depend almost entirely on these relatively low cost foods (1).

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The availability and comparatively low prices of street - vended foods relative to the processed and already - packaged foods have increased their reliability to customers (2).

Food vendors have been reported to present serious safety and health concerns to consumers. Most of them lack knowledge in safe food handling practices, environmental sanitation and hygiene. Lack of clean running water also influences the risk of contamination (3). Street vended foods or ready–to–eat foods are prone to contaminants (e. g. food borne bacteria and bio toxin) that access the food as a result of poor handling of raw materials from the farm through storage, low personal hygiene during production and preparation of the foods, and substandard practices during packaging and sale of the finished product (4). Insufficient processing and storage facilities, inadequate basic infrastructure, difficulties in regulating huge number of vendor operations, and non-permanent nature of street vending activities aggravate the public health risks posed by street vended foods (5).

Street food is a popular and convenient option for many individuals, offering a wide range of tasty snacks including puff-puff, buns, and fish roll. However, there is growing concern about the microbial quality of these street prepared food items which may pose potential health risks to consumers. Despite their popularity, street prepared puff-puff, buns, and fish roll are often prepared under uncontrolled and unsanitary conditions, with limited adherence to food safety regulations. Factors such as poor hygiene, inadequate hand washing facilities, improper storage, and lack of quality control contribute to the risk of contamination with pathogenic microorganisms. Street food vendors play significant role in providing affordable and accessible food options to urban populations. However, concerns regarding the safety and quality of street prepared food products such as puff-puff, buns, and fish roll have been raised due to the uncontrolled and often unhygienic conditions under which they are prepared and sold. This study aimed at investigating the microbial quality of street prepared snacks to assess potential health risks and provide insight for improving food safety practices in street food establishments. The study will contribute to the development of targeted training programs and guidelines for street food vendors.

2.Materials and Methods

Study area

The study was carried out within Umuahia North and South Local Government Areas of Abia State, Nigeria. The location was chosen because the area's composition was large with major activities for street food vending within the city and it is the business hub of the state. Umuahia is the capital city of Abia state in South-eastern Nigeria. Umuahia has a population of 359,230 according to the 2006 Nigeria census. Umuahia geographical coordinates are 5°32'N latitude and 7°29'E longitude (6). Umuahia is renowned for being a railway agricultural market centre which attracts traders and farmers from neighbouring towns to sell their produce. The town has a palm-oil processing plant, and the National Root Crop Research Institute Umudike located close to it (7).

Sample collection

For the purpose of this study, Umuahia which is the capital city of Abia State, Nigeria was mapped into five areas of the city (strata), Umuopara, Ibeku, Olokoro, Ubakala, and Ohuhu communities on the basis of the clans using stratified sampling. From each stratum,

puff-puff, buns, fish rolls, and swabs were randomly collected (with sterile cotton wool) using random method. A total of 25 samples collected of which 15 were snacks (puff-puff, buns, and fish roll), and 10, swabs of the production tables and surrounding floors, were packaged in sterile polythene bags and then conveyed to the Laboratory for analysis. One sample each of fish roll, buns, puff-puff, swabs from production tables and swabs from surrounding floors were further randomly selected for the microbial analyses.

Microbial analysis

Essentially, the methods described by ICMSF (9) was adopted for the determination of the total viable count (TVC), coliform count, and fungi count. Ten (10g) of each sample was weighted and transferred to a sterile blender jar where 90 mL of sterile diluent was added and blended for 1 – 2 min to create a uniform suspension. One millilitre of the homogenized suspension was transferred aseptically to a fresh 9 mL of distilled water in a separate test tube; and the serial dilution carried on to the sixth dilute (10^{-6}). The inoculum (0.1 mL) was aseptically taken from the diluents and spread evenly onto the surface of a sterile solid nutrient agar – titan biotech limited (total viable count), Sabouraud dextrose agar – HiMedia (fungi count), and MacConkey's agar – HiMedia (coliforms) medium in the plate. The inoculum plate was incubated in an incubator at 37^0C for 24 h (total viable count), 25^0C for 5 – 7 days (fungi count), and 37^0C for 24 h (coliforms). The plate was observed for microbial growth.

Identification and characterization of bacteria isolates obtained in the cultures was by means of biochemical tests which included gram's stain, catalase test, coagulase test, indole test, oxidase test, voges prokauer test, methyl blue reduction test, budding test, sporulate test, haemolysis test, glucose fermentation, lactose fermentation, sucrose fermentation, fructose fermentation, methyl red reaction, gas (CO_2) production, alcohol production, growth at 15^0C, growth at 42^0C, citrate utilization acid production and motility test (10,11).

Fungal isolates were identified and characterized on the basis of their colony and microscopic features. Colony features were observed in a pure culture of each fungal isolate which included growth characteristics, colony type and colour. Microscopic features were identified by microscopically examining fungal isolates based on their morphology and colour of the front and back surface of the colonies on the plates. The fungal identification guideline described by Samson et al. was essentially adopted (12).

Statistical analysis

Data collected from this research work were analysed using Analysis of Variance (ANOVA). Descriptive statistics in form of means and standard deviation and Duncan post hoc were used to assess the data. The Analysis were done using SPSS (Statistical Packaging for Social Science).

3.Results

Microbial Profile of Snacks

The microbial load of the snacks was assessed, TVC, fungal count, *Lactobacillus* count, *Staphylococcus count,* total coliform count, *Salmonella/Shigella* count, and *Streptococcus count*. The TVC of the snacks varied

significantly among the different types of snacks analysed. The highest TVC was observed in puff-puff, followed by fish roll, and the lowest in buns. Statistical analysis indicated that the values for each snack type were significantly different ($p<0.05$), as denoted by superscripts (c, b, a) (Table 1). The fungal load also varied across the snack types. The puff-puff had the highest fungal count, while the buns exhibited the lowest fungal count. Significant differences ($p<0.05$) were observed in the fungal counts among the snacks, as indicated by the superscripts (a, b, c) (Table 1). The *lactobacillus* count varied significantly among the snack types. Buns recorded the highest count, followed by puff-puff, and the lowest count was observed in fish roll. Statistical analysis showed significant differences ($p<0.05$) among the samples, as indicated by the superscripts (c, bc, ab) (Table 1). *Staphylococcus* was detected only in puff-puff (Table 1) while total coliforms, *Salmonella/Shigella*, and *Streptococcus* were not detected (ND) in any of the snacks analysed (puff-puff, buns, and fish roll).

Microbial profile of snacks (cfu/g)

The microbial load of the snacks was analysed for different microbial groups from two environmental sources, the service table and the floor. The TVC was higher on the Floor compared to the service table. However, no statistical significant differences ($p<0.05$) were observed between the two sources (Table 2). Fungal count was substantially higher on the floor than on the service table, although the difference was not statistically significant ($p>0.05$)

(Table 2). *Lactobacillus* was detected on the service table but not detected on the floor. The *Staphylococcus* count was higher on the service table than on the floor. Statistical analysis showed no significant difference ($p>0.05$) between the two locations (Table 2). The total coliforms were not detected (ND) on the service table but were found at significant levels on the floor. *Salmonella/Shigella* count were detected in both sources, with slightly higher counts on the floor compared to the service table. However, the difference was not statistically significant ($p<0.05$). *Streptococcus* count was slightly higher on the service table compared so the floor, with no statistically significant difference ($p<0.05$) observed.

The biochemical reactions of identified microorganisms

The biochemical characteristics of *Streptococcus lactis*, *Streptocuccus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Acebacter aceti*, and *Lactococcus lactis* were evaluated using various biochemical tests. The results are summarized in the Table 3.

The biochemical reactions revealed that: (1) *Staphylococcus aureus* was the only isolate that showed a positive slide coagulate test. (2) *Escherichia coli* displayed positive results for motility, indole reaction, and methyl red tests, while showing no fermentation of lactose or sucrose. (3) *Acetobacter aceti* and *Lactobacillus lactis* showed notable gas and acid production, with *Lactobacillus lactis* capable of growth at 15^0C and 42^0C. (4) None of the microorganisms utilized citrate or produced alcohol except for *Saccharomyces lactis* (5). Haemolysis was observed only in *Streptococcus pyogenes*.

Cultural and morphological characteristics of microbial isolates

The cultural and morphological characteristics of the microbial isolates, including *Streptococcus lactis*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Acebacter aceti*, *Lactococcus lactis*, *Aspergillus oryzae*, and *Rhizopus oligosporus*, were determined based on

colony appearance, cell structure, and other distinguishing features. The results are summarized in Table 4.

Table 1. Microbial profile of the snacks (cfu/g)

Microbial Characteristics	Snacks		
	Puff-puff	Buns	Fish roll
Total Viable Count	$60.70^{c}\times10^{6}$	$28.01^{a}\times10^{6}$	$57.58^{b}\times10^{6}$
Fungi Count	$37.35^{c}\times10^{6}$	$15.56^{a}\times10^{6}$	$31.23^{b}\times10^{6}$
Lactobacillus Count	$10.89^{bc}\times10^{6}$	$12.45^{c}\times10^{6}$	$9.34^{ab}\times10^{6}$
Staphylococcus Count	$12.45^{a}\times10^{6}$	ND	ND
Total Coliform Count	ND	ND	ND
Salmonella/Shigella Count	ND	ND	ND
Streptococcus Count	ND	ND	ND

All values are means of triplicate determinations and are significantly different at $p<0.05$ if they are in the same row but have different superscripts. Key: cfu/g = colony forming unit per gram; ND = Not Detected.

Table 2. Microbial characteristics of the processing environment (cfu/g)

Microbial Characteristics	Processing Environment	
	Service Table	Floor
Total Viable Count	$37.45^{a}\times10^{6}$	$62.25^{a}\times10^{6}$
Fungi Count	$1.55^{a}\times10^{6}$	$15.56^{a}\times10^{6}$
Lactobacillus Count	4.28×10^{6}	ND
Staphylococcus Count	$21.0^{a}\times10^{6}$	$7.78^{a}\times10^{6}$
Total Coliform Count	ND	27.23×10^{6}
Salmonella/Shigella Count	$1.55^{a}\times10^{6}$	$1.94^{a}\times10^{6}$
Streptococcus Count	$13.23^{a}\times10^{6}$	$10.24^{a}\times10^{6}$

All values are means of triplicate determinations and are significantly different at $p<0.05$ if they are in the same row but have different superscripts. Key: cfu/g (colony forming unit per gram); ND (Not Detected).

Table 3. Microbial isolates and their biochemical reactions

Biochemical Reactions	*S. lactis*	*S. pyogenes*	*S. aureus*	*E. coli*	*A. aceti*	*L. lactis*
Indole reaction	-	-	-	+	-	-
Gram reaction	+	+	+	-	+	+
Catalase reaction	-	-	+	+	+	-
Methylene blue reduction test	+	+	+	+	+	+
Motility test	-	-	-	+	-	-
Sporulate test	+	-	-	-	-	-
Budding test	+	-	-	-	-	-
Slide coagulase test	-	-	+	-	-	-
Voges proskeur	-	-	-	-	-	-
Haemolysis test	-	+	-	-	-	-
Glucose fermentation	+	-	+	-	+	-
Lactose fermentation	+	-	-	+	-	+
Sucrose fermentation	+	-	-	-	+	-
Fructose fermentation	+	-	-	-	+	-
Methyl red reaction	+	-	-	+	-	-
Gas (CO_2) production	+	-	-	+	+	+
Acid production	-	-	-	+	+	+
Alcohol production	+	-	-	-	-	-
Growth at 15^0C	-	-	-	-	-	+
Growth at 42^0C	-	-	-	-	-	+
Citrate utilization	-	-	-	-	-	-

Key: A. aceti (Acetobacter aceti); L. lactis (Lactobacillus lactis); E. coli (Escherichia coli); S. aureus (Staphylococcus aureus); S. pyogenes (Streptococcus pyogenes); S. lactis (Saccharomyces lactis); + (Positive); - (Negative)

Table 4. Microbial isolates and their cultural and morphological characteristics

Morphological and Cultural Characteristics	*S. lactis*	*S. pyogenes*	*S. aureus*	*E. coli*	*A. aceti*	*L. lactis*	*A. oryza*	*R. oligosporus*
Flagella	-	-	-	+	+	-	-	-
Oval and roundish cells	+	+	+	-	-	-	-	-
Rod shaped cells	-	-	-	+	+	+	-	-
Filamentus branching cells	-	-	-	-	-	-	+	+
Cells in oval chains	-	+	-	-	-	+	-	-
Cells in clusters	+	-	+	-	-	+	-	+
Whytish colonies	+	+	-	+	+	+	+	+
Golden yellow colonies	-	-	+	-	-	-	+	-
Brownish colonies	-	-	-	-	-	+	-	-
Budding cells present	+	-	-	-	-	-	-	-
Colonies with smooth edges	+	+	+	+	+	+	+	-
Colonies with wrinkled edges	-	-	-	-	-	-	-	+
Spores and sporangia	-	-	-	-	-	-	+	+
Stolena	-	-	-	-	-	-	-	+
Rhizoids	-	-	-	-	-	-	-	+
Conidia & conidiophores	-	-	-	-	-	-	+	+
Septea (Crosswalls)	-	-	-	-	-	-	+	-
Phialids	-	-	-	-	-	-	+	-
Vesicles	-	-	-	-	+	-	-	-
Aerial mycelia	-	-	-	-	-	-	+	+
Metallic sheen colonies	-	-	-	-	-	-	-	-

Key*: A. oryza (Aspergillus oryza); R. oligosporus (Rhizopus oligosporus); S. lactis (Saccharomyce lactis); S. pyogenes (Septococcus pyogenes); S. aureus (Stapylococcus aureus); A. aceti (Acetobacter aceti); L. lactis (Lactobacillus lactis); E. coli (Escherichia coli);* + (Positive); - (Negative); NA (Not Applicable).

The cellular morphological characteristics were observed in *Saccharomyce lactis*, *Septococcus* pyogenes, and *Stapylococcus aureus* (for Oval and roundish cells), *Escherichia coli*, *Acetobacter aceti*, and *Lactobacillus lactis* (for rod-shaped cells), as well as *Aspergillus* oryzae and *Rhizopus* oligosporus (for filamentous branching cells). Colony characteristics including whitish colonies were present in all isolates except *Stapylococcus aureus* whereas golden yellow colonies were noted for *Escherichia coli* and *Acetobacter aceti*, while brownish colonies were observed in *Lactobacillus lactis*. Special structures namely, spores, sporangia, stolons and rhizoids were specific to *Aspergillus* oryzae and *Rhizopus oligosporus* as well as conidia and conidioshores, along with septa and phialids, which were also observed in *Aspergillus oryzae*. All isolates except *Rhizopus oligosporus* produced colonies with smooth edges, while *Rhizopus oligosporus* alone formed colonies with wrinkled edges.

4.Discussion

Microbial assessment of the snacks

The microbial profile of snacks (puff-puff, buns, and fish roll) collected within Umuahia town in Abia state is shown in **Table 1.** These results indicate a high level of microbial contamination in the street-vended puff-puffs, buns, and fish rolls in Nigeria. The TVC and fungi count exceeded recommended limits, indicating a high risk of foodborne illness. The presence of *Staphylococcus aureus* in puff-puffs is particularly concerning, as it can cause food poisoning. All samples had alarmingly high TVC which exceeded the recommended limit of $\leq 10^5$ cfu/g for ready-to-eat foods (13, 9). This high load of TVC indicates severe microbial contamination which could be due to poor hygiene, poor storage conditions or a neglect of overall safety protocol. Their Fungi Count also exceeded the recommended limit of $\leq 10^2$ cfu/g (14) indicating a high level of microbial contamination. Fungal contamination is a common issue in street-vended foods in Nigeria (15, 17). All samples have moderate levels of *Lactobacillus*, which is within the expected range for fermented foods. Puff-puff exceeds the recommended limit of $\leq 10^5$ cfu/g. *Staphylococcus aureus* exceeds acceptable limits of $\leq 10^4$ cfu/g (18). *Staphylococcus aureus* is a common foodborne pathogen, and its presence is a concern (19) as eating these foods can lead to foodborne illnesses, such as gastroenteritis, diarrhea, and vomiting. High levels of *Staphylococcus aureus* can cause food poisoning, which can be severe and even life-threatening. Vulnerable people such as the elderly, pregnant women, and children, are more susceptible to foodborne illnesses. However, all samples had low or undetectable levels of *coliform, Salmonella* and *Streptococcus* species which is a positive finding. In general, the prevalence of *Staphylococcus aureus* in puff-puff in significant numbers is of great concern. S*taphylococcus* species is implicated in most common food borne infections especially when not prepared and handled aseptically or hygienically as the case is in these snacks. They are often left uncovered at the mercy of flies, dust, and smoke from exhaust pipes of cars and motorcycles, and buyers may be allowed to freely touch and select with bare hands. These results are in agreement with those of (20).

Microbial assessment of the production environment

The results of the microbial assessment of the production and sales environment are shown in Table 2. The results indicate a highly contaminated production environment for puff-puff, buns, and fish rolls, posing significant food safety risks.

Of all the microbial characteristics assessed, only *Lactobacillus* count (floor) and total coliform count (service table) remained below detectable limits. All other characteristics were quite high. High TVC indicate significant microbial presence reflecting poor sanitation and potential for contamination. Low TVC generally below $\leq 10^4$ cfu/g is expected in production areas if standard guidelines are followed to avoid microbial contamination of food products. Elevated fungi count as seen in this study suggests mould growth and mycotoxin production which can lead to spoilage and is a health risk. *Staphylococcus aureus* is a significant foodborne pathogen that can produce toxins causing food poisoning. Levels should be undetectable in foods, but these high counts are alarming and indicate severe hygiene issues. Coliform bacteria are indicators of faecal contamination, hence total coliform count indicates poor sanitary conditions. High counts necessitate improvement in cleanliness. *Salmonella/Shigella* plate count occurred in mild load on both surfaces which agrees with the report of (21). However, their presence in any count is unacceptable in food production areas as these organisms are severe foodborne pathogens posing a risk of salmonellosis and shigellosis. The result also gave an indication that there could occur a prevalence of *salmonellosis* and typhoid fever in these locations. Still, the presence of these organisms indicates poor food preparation and practices (7). High counts of *Streptococcus spp.* indicate potential streptococcal contamination and poor hygiene necessitating improved sanitation practices (8). These results suggest a high risk of foodborne illness due to poor hygiene and contamination of the production environment.

Microbial identification by biochemical reaction

Table 3 shows result obtained when inoculates were subjected to a series of biochemical tests ranging from gram reactions to citrate utilization test. The organisms, *Saccharomyces lactis, streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, Acetobacter aceti, Lactobacillus lactis,* and fungi isolates, were all confirmed present. Similar types of microbial contaminants were identified in previous studies in Benin City, Ogun State and Ondo State all in Nigeria (22, 23). The presence of these organisms in these locations indicates that there could be possible outbreak of bronchitis, *staphylococcus* infection and gastroenteritis.

Microbial identification by morphological and cultural characteristics

Table 4 shows the result of microbial identification by analysis of the morphological and cultural characteristics. *Aspergillus oryza* as well as *Rhizopus oligosporus* were identified by this method in addition to the organisms in Table 3 identified via the biochemical method. From the list of organisms identified in both tables, only *Acetobacter aceti, Saccharomyces lactis,* and *Lactobacillus lactis* were not considered pathogenic but safe and beneficial in food production, even though their presence still indicates contamination or poor sanitation practices. All others are considered food safety hazards: *Aspergillus oryzae* produces mycotoxins potentially causing allergic reactions and respiratory issues; *Rhizopus oligosporus* causes gastrointestinal infections and allergic reactions; *Streptococcus pyogens* causes streptococcal toxic shock syndrome and necrotizing fasciitis; *Staphylococcus aureus* produces enterotoxins, causing food poisoning

and potentially life-threatening infections; *Escherichia coli* apart from being a coliform an indication of faecal contamination and unhygienic practices, causes food poisoning, urinary tract infections, and potentially life-threatening conditions. The presence of these organisms in food is indicative of food vendors being carriers of pathogenic microorganisms (24). These organisms are also listed by the International Commission on Microbiological Specifications for Foods (ICMSF) as harmful and must be controlled through proper sanitation and hygiene practices (25).

5.Conclusion

The study indicates poor microbial quality of the street-vended puff-puffs, buns, and fish rolls with high levels of bacterial and fungal contamination. It also indicates a highly contaminated production environment posing significant risks of food borne illness. The presence of *Staphylococcus aureus* in snacks (puff-puffs) is a concern, as it can cause foodborne illness. The low levels of *coliform*, *Salmonella/Shigella*, and *Streptococcus* spp. are a positive finding, but the overall high microbial load suggests a need for improved handling, storage, and hygiene practices to ensure food safety. Education of the food vendors and consumers on the risks of selling and eating respectively contaminated foods as well as regular monitoring and enforcement of food safety regulations are recommended.

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Author contribution

The authors worked collaboratively to ensure a successful delivery of the project. The contributions of Abasiekong, Kuyik Solomon include conceptualization, methodology, supervision, writing original manuscript, review and editing. Those of Nwanagba, Nkiruka Lilian were supervision, conceptualization, and critical revision of the manuscript. Orji, Adaobi Mary contributed in the areas of investigation, data curation, project administration, writing of original draft.

Declaration of competing interest

Authors have no known financial and personal relationships with other people or organizations that could inappropriately influence or bias their work.

Data availability

The datasets used and/or analysed during the study are available from the corresponding author upon reasonable request.

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