MENTORSHIP, SCHOLARSHIP, AND SERVICE: A SPECIAL ISSUE HONOURING PROFESSOR ADAMU N. BABA-KUTIGI, FNIP ON HIS RETIREMENT

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Microbiological Quality Assessment of Ready-To-Eat Snacks Sold in Selected Primary Schools in Ovia North-East Local Government Area of Edo State, Nigeria

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ABSTRACT

Ready-To-Eat (RTE) snacks prepared under unsanitary conditions and sold by unlicensed vendors at improper holding temperatures, without appropriate packaging, poses a health risk to consumers. This study is aimed at evaluating the microbiological quality of RTE snacks sold within the premises of three (3) selected primary schools coded 'ADPS', 'OPS', and 'IPS' in Ovia North-East LGA of Edo State, Nigeria. A total of thirty-nine (39) samples that comprise of 10 buns, 10 doughnuts, 10 sausage roll, and 9 meat pies, were randomly purchased from vendors around the school premises using aseptic techniques. Microbiological analyses of the samples were carried out using culture media, following standard procedures. Identification of the isolates was based on cultural, morphological, and biochemical characteristics. Antibiotic susceptibility pattern of isolates were determined using Kirby Bauer disk diffusion method. Mean percentage occurrences of microbial contaminants in the snacks are *Staphylococcus aureus* (25.64%), *Enterococcus* sp. (12.82%), *Bacillus* sp. (12.82%), *Aspergillus* sp. (12.82%), *Pseudomonas* sp. (10.26%), *Fusarium* sp. (10.26%), *Escherichia coli* (7.69%), and *Penicillium* sp. (7.69%). Mean total aerobic plate counts (APC) ranged from 0.8×10^2 - 1.16×10^6 CFU/g. based on the International Commission for Microbiological Specification for Foods (ICMSF) for APC, the snack samples from ADPS was satisfactory. Lowest percentage of the isolates (48.72%) that demonstrated antibiotic resistance was against ceftazidime, while the highest (71.79%) involved nitrofurantoin. To avert the outbreak of food borne diseases, strict monitoring of hygienic and sanitary practices of snack producers and vendors by the school management and relevant authorities are recommended.

Keywords: Antibiotic Resistance, Foodborne Diseases, Food Safety, Hygiene, Snacking, Vendors

INTRODUCTION

Globally, it is estimated that 125, 000 children not exceeding 5 years die prematurely every year as a result of consuming contaminated food and water (WHO, 2016). The total number of lives lost due to food borne illnesses is 420, 000 annually (Moloi et al., 2021; Maduka & Ugbogu, 2024). Food sanitary quality controls put in place to reduce the incidence of food borne diseases by relevant agencies in Nigeria is weakly implemented (Salihu & Salihu, 2022). Food safety is universally recognized as the right of every individual because human health is affected when unsafe food is consumed (Olisaka et al., 2021). In addition to the food prepared at home which some pupils carry to school, others rely on RTE foods and snacks sold within the school premises and its environs to sustain them during school hours (Maduka et al., 2022).

RTE snack is a category of food that does not require cooking or processing before it is consumed. Snacks are regarded as safe for human consumption. Its ready availability, affordability, and convenient to eat are key factors responsible for wide acceptability of snacks (Alhalabi et al., 2024). The definition of snack stated in the Guidelines for the Microbiological Examination of RTE foods excluded nuts that have shell, whole fruits and vegetables in its raw form that requires hulling, peeling or washing before they are consumed (Okeke et al., 2021). In recent years, snacking has become a common habit associated with modern lifestyle in the cities and semi-urban settlements. Snacking is a common habit among children, youths, and adults of different age groups, culture, religion, and social status (Ahaotu et al., 2022). Urbanization, liberalization, globalization, among other factors have significantly contributed to changes in snacking pattern observed among greater population of residents in big cities (Akkavva et al., 2019). Producers of snacks target children by making available products of different sizes, shapes, colours and flavours appealing to them (Alhalabi et al., 2024). The level of consumption of snacks among children and youths is very high in Nigeria (Okeke et al., 2021).

Meat pie is a widely consumed snack. It is a filled baked product prepared using wheat flour, eggs, sugar, vegetable oil, beef, potatoes especially *Solanum tuberosum*. Beef and potatoes are commonly used as filler to prepare meat pie. The filler is properly covered by flour dough. Different savoury ingredients depending on individual choice, eggs, and fish can also be used as filler for preparing meat pie (Eluma et al., 2015). Although filled baked products such as meat pie is nutritious, the shelf life of the product is shorter compared with several snacks. The nutrients in meat pie encourage rapid multiplication of food spoilage microorganisms (Falola et al., 2011; Okechukwu et al., 2016).

Sausage is another snack 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 baked in a 350 degrees Fahrenheit preheated oven for 30 to 35 minutes. Puff puff is made from yeast dough containing eggs, butter, water, flour, yeast and sugar shaped into balls and deep-fried until it turns golden brown. Doughnut is basically made of wheat flour and eggs creamed with butter and sugar. It is fried using hot vegetable oil or baked inside an oven (Okeke et al., 2021).

The number of individuals and businesses owners involved in producing and selling snacks is increasing in many cities and towns in Nigeria. Many of them are poorly educated, unlicensed, and untrained in food hygiene practices. They usually conduct their businesses in unsanitary conditions and have little knowledge about food borne diseases and preventive measures (Falola et al., 2011; Ike et al., 2015). Due to high level of poverty, illiteracy, and unemployment in the country, a lot of children, youths, and adults have been forced into producing and hawking affordable snacks as a means of survival, with less emphasis on food safety (Nicolas et al., 2007). Commercially available RTE snacks are produced without proper supervision and strict compliance to food safety guidelines. These snacks are poorly packaged and sold to consumers in unhygienic environments where houseflies, fruit flies, air, and dust are potential sources of contamination (Ngueugang et al., 2021).

At various stages of preparing snacks and product handling, it is predisposed to microbial contamination. Snack exposed to high level contamination and sold to the general public increases the risk of food borne disease outbreak especially among children, elderly, and immunocompromised (Okeke et al., 2021). Different bacterial species were isolated from samples of RTE foods sold in parts of Benin City, Edo State (Oshoma et al., 2019).Some of these bacterial species could harbour antibiotic resistance genes which usually spread among microorganisms that could infect human population. Consequently, disease conditions could manifest and death is reported in extreme conditions (Ogidi et al., 2016).

Several studies on quality assessment and health risks associated with consumption of RTE foods sold in the streets and various retail outlets located in different towns have been reported (Ogidi et al., 2016; Imathiu, 2017; Nemo et al., 2017; Obueh et al., 2017; Obaji et al., 2018; Ibrahim et al., 2020; Olisaka et al., 2021; Compaore et al., 2022; Izah et al., 2022). Despite the popularity of RTE snacks, limited studies on its microbiological quality have been carried out. On a daily basis, many primary school pupils and their teachers in Ovia North-East, are at risk of consuming unwholesome RTE snacks sold within the school premises and its environs. Therefore, this study is aimed at assessing the microbiological quality and antibiotic susceptibility pattern of bacterial and fungal species isolated from popular snacks which include buns, doughnuts, and meat pie sold within the premises of selected primary schools in Ovia North-East Local Government Area, Edo State, Nigeria.

MATERIALS AND METHODS

Study Area

The headquarters of Ovia North-East Local Government Area (LGA) is Okada town. The size of the Ovia North-East LGA situated in Benin City, capital of Edo State, is 2, 301 square kilometer. Geographically, it is bounded by longitude 5º 45' and 6º 15' east and latitude 5º 15' and 6º 45' north of the central province of Edo State. A major river in the LGA is known as Ovia river which passes through every community (Rawlings & Ikediashi, 2020). According to Ikuenobe & Olubor (2019), Ovia North-East LGA has a total of eighty-six (86) public primary schools. Presented in Figure 1 is the map of Ovia North-East and other Local Government Areas of Edo State, Nigeria.

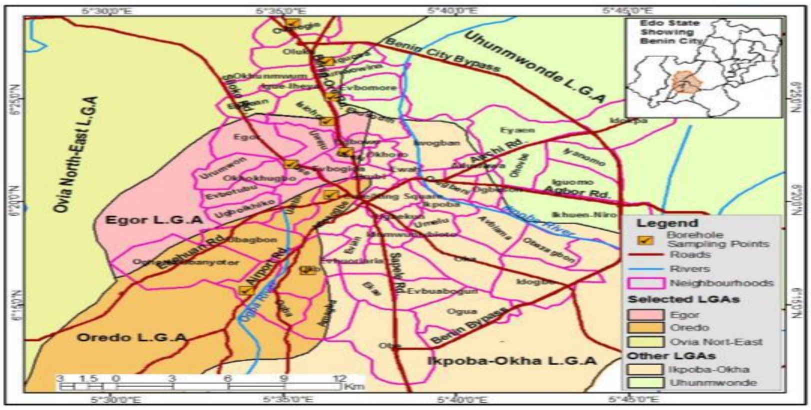

Figure 1: Map of Ovia North-East and other Local Government Areas of Edo State.
Source: Rawlings & Ikediashi (2020).

Sample Collection

A total of thirty-nine (39) samples of RTE snacks were obtained from vendors inside the premises of three (3) selected primary schools in Ovia North-East LGA, Edo State. Snack samples were purchased from four (4) randomly selected vendors in each primary school. The snacks comprise 10 buns samples, 10 doughnut samples, 10 sausage samples, and 9 meat pie samples. The samples were collected between 11:00 am and 12:30 pm, which coincided with the break time for the school children. The snack samples were collected aseptically using a sterile polyethylene bags and immediately transported to the laboratory for microbial analysis.

Serial Dilution and Microbiological Analyses

Each snack sample was crushed using a mortar and pestle sterilized with 70 % ethanol. Exactly 25 g of each sample was homogenized with 225 ml sterile buffered peptone water. Serial dilution of stock solution of each sample was carried out until 10^{-6} was reached. Ten-fold serial dilution of the stock solution was carried by aseptically transferring 1 ml solution into 9 ml sterile peptone water using sterile pipette. Stepwise transfer of the solution into subsequent test tubes containing 9 ml sterile peptone water was carried out using sterile pipette for each transfer until 10^{-6} dilution was reached. Using the pour plate method, 0.1 ml dilution of each snack sample was inoculated into nutrient agar (for aerobic plate count), MacConkey agar (for enumeration of coliform/enteric organisms), Eosin methylene blue agar (for *Escherichia coli* enumeration), Mannitol salt agar (for enumeration of *Staphylococcus aureus*) and Potato dextrose agar incorporated with 5% chloramphenicol (for enumeration of fungi) in triplicates. All the media used were prepared following manufacturer's instruction and sterilized at 121 °C for 15 minutes at 15 psi. The inoculated plates were incubated at 37 °C for 24 - 48 h for isolation of bacteria, while fungi involved room temperature (27±1 °C) incubation for 5 - 7 days. Colonies that appeared on the culture plates were counted using the illuminated colony counter. The total aerobic plate count, total coliform count, *E. coli* count, *S. aureus* count, and total fungal counts of each sample was expressed as colony forming unit per volume (CFU/ml) using the following formula in equation 1 (Ire & Imuh, 2016; Kabiru & Madaki, 2017).

$$\text{CFU/ml} = \text{No. of colonies} \times \frac{1}{Serial\ dilution} \times \frac{1}{Dilution\ plated} \quad (1)$$

Obtaining Pure Isolates

After incubation of the culture plates, visible bacterial colonies were sub-cultured into freshly prepared media which include nutrient agar (NA), MacConkey agar (MCA), Eosin methylene blue (EMB) agar, and Mannitol salt agar (MSA). The pure isolates were stored on slants until proper identification was completed which involves Gram staining, motility and biochemical tests. These tests were performed on each discrete bacterial colony obtained for each snack sample (Ngueugang et al., 2021).

Cultural, Morphological and Biochemical Characterization of the Isolates

The Bergey's Manual of Determinative Bacteriology was used to identify the bacterial isolates based on the result obtained from Gram stain reaction and biochemical tests. Colony appearance of each isolate in the culture plate was noted. Gram staining, followed by microscopic examination of the stained organism revealed its morphological characteristics and group of bacteria it belongs (Gram positive or Gram negative). The biochemical tests carried out on the bacterial isolates include catalase, indole, urease, oxidase, coagulase, citrate, Voges- Proskauer, and sugar fermentation tests. Macroscopic identification of fungal isolates was performed using lactophenol cotton blue (Isu & Onyeagba, 2002; Shoaib et al., 2020).

Antibiotic Susceptibility Tests

Antibiotic susceptibility tests were carried out on the bacterial isolates using the Kirby-Bauer disk diffusion method (Bauer et al., 1966).Aliquot of discrete colonies were suspended in nutrient broth and incubated for 4 h at 37°C. The turbidity of the broth culture was adjusted and compared with 0.5 McFarland turbidity standards (1.5 x 10^8 CFU/mL). The isolates were inoculated onto freshly prepared Mueller-Hinton agar (MHA) for bacterial species, while potato dextrose agar (PDA) is for fungal species. A sterilized forceps was used to lift the antibiotic disc from the manufacturer's pack (Oxoid) and placed on MHA and PDA inoculated plates. The impregnated antibiotic discs were allowed to diffuse into the agar before the plates were incubated for 24 h at 37 ºC and 5 days at room temperature for bacterial and fungal isolates, respectively. The diameter of zone of inhibition around each antibiotic in the disk was measured to the nearest millimeter using a vernier caliper. Zone diameter ≥ 16 mm was interpreted as sensitive, while a zone ≤ 13 mm was considered as resistant. Each measurement was taken in triplicates and the average calculated (CLSI, 2006).

Statistical Analysis

All the analyses were performed in triplicates. The mean and standard deviation of the values for each analysis was calculated.

RESULTS AND DISCUSSION

Table 1 shows the mean total aerobic plate count and total fungal count of snack samples obtained from vendors operating within the premises of three primary schools coded ADPS, OPS, and IPS. The result shows that the doughnut samples obtained from vendors in OPS (7.8± 0.1 x 10^5 CFU/g) and ADPS (<1 CFU/g) had the highest and lowest aerobic plate count, respectively. Meat pie obtained from OPS (2.6 ± 0.1 x 10^2 CFU/g) represent the highest fungal count, whereas all the RTE snack samples from ADPS had the least (<1 CFU/g) values.

Table 1: Mean Total Aerobic Plate Counts and Total Fungal Counts (CFU/g)

Selected school code	Snack	Fungal counts	Bacterial counts
ADPS	Buns	<1	8.0 ± 0.1 x 10^1
	Doughnut	<1	<1
	Sausage	<1	1.5 ± 0.1 x 10^2
	Meat pie	<1	2.2 ± 0.2 x 10^2
OPS	Buns	1.3± 0.2 x 10^1	1.4 ±0.1 x 10^2
	Doughnut	1.7 ± 0.1x 10^2	4.7 ± 0.1 x 10^2
	Sausage	6.0± 0.2 x10^1	2.8 ± 1.1 x 10^2
	Meat pie	2.6 ± 0.1x 10^2	1.16 ± 0.5 x 10^6
IPS	Buns	1.1 ± 0.2x 10^2	4.1 ± 0.3 x 10^2
	Doughnut	9.0 ± 0.1 x 10^1	7.8 ± 0.1 x 10^5
	Sausage	1.0 x 10^1 ± 0.1	5.7 ± 0.1 x 10^2
	Meat pie	1.4 ± 1.1x 10^2	4.0 ± 0.2 x 10^2

In Lagos state, Okeke *et al*. (2021) reported that the maximum mean bacterial count of meat pie and doughnut samples obtained from four locations is 1.50 x 10^6 and 3.20 x 10^5 CFU/g, respectively. Omorodion (2022) reported that the mean total viable count of meat pie and doughnut sold in two fast food restaurants and two road side vendors around the University of Port Harcourt is within the range of 3.08 - 4.66 log_{10}CFU/g and 3.32 - 6.38 log_{10}CFU/g, respectively. In a related study, Effiong et al. (2024) reported that the total heterotrophic bacterial count of meat pie obtained from selected stores in Hezekiah University is 4.41 log_{10}CFU/g. The bacterial isolates that contaminated the meat pie were *Bacillus* sp. and *Escherichia coli*.

Among the three selected primary schools where RTE snacks were sampled, the product obtained from vendors within ADPS had the lowest mean aerobic plate count (1.5±0.1 x 10^2 CFU/g) interpreted as satisfactory based on the International Commission for Microbiological Specification for Foods (ICMSF). Only *Staphylococcus aureus* was isolated from the snack samples obtained from ADPS. This result could be attributed to high level personal hygiene and good manufacturing practices (GMPs) implemented by producers of the RTE snacks. The use of appropriate packaging materials might have contributed in reducing the level of microbial contamination of the snacks. The school management in ADPS might have rules and regulations that effectively monitor the activities of snack vendors within their premises in the interest of consumers.

Presented in Table 2 are the morphological characteristics, biochemical, and motility tests of bacterial isolates obtained from the snack samples. Five (5) genera of bacteria were isolated from the samples. They include *Escherichia coli, Enterococcus* sp., *Pseudomonas* sp., *Staphylococcus aureus* and *Bacillus* sp. In a related study, Okeke et al. (2021) isolated *Bacillus subtilis* and *B. cereus* from meat pie samples sold in parts of Lagos State, while doughnut samples obtained from the city were also contaminated with *Staphylococcus aureus*, *Bacillus subtilis*, and *B. cereus*. According to Ahaotu et al. (2022), *Staphylococcus aureus* is a normal flora of the human and animal skin. The bacterium is also present in the nasal passage and throat. Excessive handling of the RTE snacks by the producers and vendors could be a source of contamination. This could be one of the reasons why *S. aureus* encountered in the RTE snacks had the highest percentage occurrence (25.64%) compared with other isolates. Sneezing and blowing the nose by sellers and buyers of RTE snacks very close to where the product is displayed could lead to contamination by *S. aureus*. For easy opening of polyethylene bags to put snacks, some sellers have formed the habit of using their mouth to blow air on it. By so doing, the snack is likely to be contaminated with *S. aureus* (Okeke et al., 2021). Polyethylene bags used in packaging snacks is a possible source of microbial contamination of snacks. It is important to note that certain strains of enterotoxigenic *Staphylococcus* are implicated in food borne diseases. Tahir et al. (2016) reported that buns sold in parts of Wuntin Dada Area, Bauchi state was contaminated with *Staphylococcus aureus* and *Bacillus cereus,* while *S. aureus* was reported in meat pie.

Table 2: Morphological and Biochemical Characteristics of Bacterial Isolates

Morphology, biochemical and motility tests	**Isolate 1**	**Isolate 2**	**Isolate 3**	**Isolate 4**	**Isolate 5**
Cultural elevation	Convex	Slightly raised	Umborate	Convex	Raised
Margin	Entire	Irregular	Entire	Entire	Entire
Colony	Slightly mucoid	Distinct	Mucoid	Smooth	Smooth
Colour	Greyish white	Dark Red	Greenish pigment	Golden Brown	Yellow
Shape	Rod	Circular	Rod	Circular pinhead	Circular
GramStain Reaction	-	+	-	+	+
Cell arrangement	Rod	Cocci	Rod	Cocci	Rod
Catalase	+	-	-	+	+
Indole	+	NA	-	-	-
Urease	+	NA	+	+	-
Oxidase	-	NA	+	-	-
Coagulase	-	-	-	+	-
Citrate	-	+	+	+	-
Voges Proskauer	-	+	NA	-	NA
Mannose	+	+	NA	+	NA
Glucose	+	+	NA	NA	NA
Sucrose	-	+	NA	+	NA
Motility	+	-	+	-	+
Probable organism	*Escherichia coli*	*Enterococcus* sp.	*Pseudomonas* sp.	*Staphylococcus aureus*	*Bacillus* sp.

Key: NA = Not applicable; - represent negative; + represent positive.

Table 3 shows the cultural and morphological characteristics of the fungal isolates from the RTE snacks. The fungi contaminants were identified as *Penicillium* sp., *Fusarium* sp., and *Aspergillus* sp. These fungal species are associated with bakery products often consumed as snacks. With the exception of *Fusarium* sp., others are often implicated in the spoilage of bakery products. Possible sources of contamination of the snacks by these fungal species are soil and plants. The presence of some *Aspergillus* species in baked products could be attributed to the ability of the fungus to resist heating that usually takes place during baking (Ahaotu et al., 2023).

Table 4 shows the frequency/mean percentage occurrence of bacteria and fungi isolated from the RTE snack samples. The result shows that *Staphylococcus aureus* had the highest mean percentage occurrence (25.64%). However, the least involved *Escherichia coli* (7.69%) and *Penicillium* sp. (7.69%).

The occurrence of *Escherichia coli* in the RTE snacks is an indication that fecal contamination from animal and humans took place during preparation and handling of the product. *Bacillus* sp. is a spore former capable of surviving in harsh environmental conditions. Its ubiquitous status is well-reported. Two bacterial genera which include *Bacillus* spp. (12.82%) and *Enterococus* spp. (12.82%) also isolated from the RTE snacks had the same percentage occurrence. According to Ahaotu et al. (2021), *Bacillus* spp. release toxins implicated in food poisoning syndromes referred as diarrhea and emesis. Therefore, the presence of the bacterium in the snack samples poses a health risk to consumers.

Among the three RTE snack samples obtained from the vendors, only meat pie was not contaminated with *Pseudomonas* sp. This result is not in agreement with the report by Omolaja & Adeleke (2013). The researchers reported the presence of *Pseudomonas* sp. and other bacterial species in the meat pie samples sold in selected eateries and local kiosks in Ogun State. A similar result was reported by Clarence et al. (2009) in the meat pie samples sold in Benin City, Nigeria. Soil and water are possible sources of *Pseudomonas* sp. isolated from the snack samples. The bacterium plays a role in the spoilage of food samples. It is also implicated in gastrointestinal infections (Maduka & Ugbogu, 2024).

The presence of *Enterococcus* sp. in the RTE snacks is an indication of poor hygiene among the handlers and producers of the product. Many strains of *Enterococcus* could cause opportunistic infections in humans. A recent study by Moro et al. (2024) in fast food outlets in Lagos, reported that *Enterococcus faecalis* (67.9%) was isolated from palms of food handlers, *E. gallinarium* (21.4%) from food samples, while table tops were contaminated with *E. faecium* (10.7%). The source of *Pseudomona*s spp. in the RTE snacks could be from the environment, soil, water, dirty utensils, and food handlers. The bacterium is capable of causing gastroenteritis in humans. Its role in food spoilage has also been reported (Olisaka et al., 2021).

Table 3: Cultural and Morphological Characteristics of Fungal Isolates

Isolates	Cultural characteristics	Morphological Characteristics	Probable organism
Isolate 1	Large fluffy white colonies almost covering the whole surface	Non-septate branched hyphal enlarged at the apex to form conidophore. They produce brownish black conidia in chains.	*Penicillium* sp.
Isolate 2	Rapidly growing wooly to colt only lemon and yellow	Multicellular distinctive sickle shaped macro conidia.	*Fusarium* sp.
Isolate 3	Very common colors of colony (black and white)	Conidia borne in 360 arrangements covering the upper 2/3 of the conidiophores	*Aspergillus* sp.

In a related study, Amadi et al. (2014) isolated *Aspergillus flavus, A. fumigatus, Fusarium solani, F. oxysporum, Penicilium digitatum* and *Rhizopus* sp. from meat pie, buns and doughnut sold in different locations in Nnamdi Azikiwe University campus and its environs. According to Okeke et al. (2021), doughnut samples obtained from parts of Lagos state were contaminated with *Aspergillus niger*, *Penicillium notatum*, *Trichoderma* spp. and *Saccharomyces cerevisiae*, while meat pie samples involved *Fusarium* sp., *Aspergillus niger*, *Saccharomyces cerevisiae* and *Trichoderma* spp. *Aspergillus* sp. are commonly found in different environments. Its presence in food is a source of concern because some *Aspergillus* sp. is capable of producing mycotoxins such as Aflatoxins B_1, B_2, G_1 and G_2. *Penicillium* sp. is widely distributed in nature. Moniliformins is a toxin produced by *Fusarium* sp. The presence of *Penicillium* sp. in the RTE snacks is a bit worrisome because it is associated with food spoilage. Infection caused by *Penicillium* sp. in humans especially immunocompromised is known as penicilliosis (Ahaotu et al., 2022; Ahaotu et al., 2023).

Depicted in Table 5 is the bacterial and fungal species isolated from each of the RTE snacks. The result shows that 4 out of 5 bacterial species obtained from all the snacks were present in buns and doughnut obtained from OPS and IPS, respectively. Only doughnut obtained from ADPS had no culturable bacterial species. A similar result with regards to fungal species was reported in all the RTE snacks obtained from ADPS. At least, one fungi species was reported in other snacks obtained from two (2) primary schools.

Table 6 shows the bacteriological quality of RTE snacks obtained from three primary schools (ADPS, OPS, and IPS). Snack samples obtained from ADPS were satisfactory, whereas the sample from OPS was not.

Table 4: Percentage Occurrence of Bacteria and Fungi Isolated From the RTE Snack Samples

Microbial Isolates	Sample Buns	Doughnut	Sausage	Meat pie	Frequency	Mean percentage occurrence (%)
Escherichia coli	0 (0.00)	0 (0.00)	1 (10.00)	2 (22.22)	3	7.69
Enterococcus sp.	1 (10.0)	1 (10.00)	2 (20.00)	1(11.11)	5	12.82
Pseudomonas sp.	2 (20.00)	1(0.00)	1 (10.00)	0 (0.00)	4	10.26
Staphylococcus aureus	4 (40.00)	2 (20.00)	2 (20.00)	2 (22.22)	10	25.64
Bacillus sp.	1 (10.00)	2 (20.00)	1 (10.00)	1(11.11)	5	12.82
Penicillium sp.	0 (0.00)	1 (10.00)	1 (10.00)	1 (11.11)	3	7.69
Fusarium sp.	1 (10.00)	1 (10.00)	1 (10.00)	1 (11.11)	4	10.26
Aspergillus sp.	1 (10.00)	2 (20.00)	1 (10.00)	1 (11.11)	5	12.82
Total number of isolates	10 (25.64)	10 (25.64)	10 (25.64)	9 (23.08)	39	100.0

Table 5: Bacterial and Fungal Species Isolated From RTE Snacks

Selected Schools	RTE Snacks	Fungal Species	Bacteria Species
ADPS	Buns	None	*Staphylococcus aureus*
	Doughnut	None	None
	Sausage	None	*Staphylococcus aureus*
	Meat pie	None	*Staphylococcus aureus*
OPS	Buns	*Aspergillus* sp.	*Pseudomonas* sp., *Bacillus* sp., *Enterococcus* sp., *Staphylococcus aureus*
	Doughnut	*Penicillium* sp., *Aspergillus* sp.	*Bacillus* sp., *Staphylococcus aureus,*
	Sausage	*Aspergillus* sp.,	*Enterococcus* sp., *Pseudomonas* sp.
	Meat pie	*Fusarium* sp. *Penicillium* sp.	*Escherichia coli, Enterococcus* sp., *Bacillus* sp.
IPS	Buns	*Fusarium* sp.	*Pseudomonas* sp., , *Staphylococcus aureus*
	Doughnut	*Fusarium* sp., *Aspergillus* sp.	*Bacillus* sp., *Pseudomonas* sp., *Enterococcus* sp., *Staphylococcus aureus*
	Sausage	*Penicillium* sp.	*Staphylococcus aureus, Escherichia coli, Enterococcus* sp.
	Meat pie	*Aspergillus* sp.	*Escherichia coli, Staphylococcus aureus*

Table 6: Bacteriological Quality of RTE Snacks Obtained From Three Primary Schools

Selected Schools	Bacterial Counts (n=39)	Mean values (CFU/g)	Aerobic plate count (ICMSF, 2016)
ADPS	8.0 x 10^1- 2.2 x 10^2	1.5 ± 0.1 x 10^2	Acceptable (0 - 10^3)
OPS	1.4 x 10^2 - 1.16 x10^6	5.8± 1.0 x10^5	Unsatisfactory (>10^6)
IPS	4.0 x 10^2 - 7.8 x10^5	3.9± 0.2 x10^5	Tolerable (10^4 - >10^5)

The antibiotic resistance pattern of bacterial and fungal species isolated from RTE snacks is presented in Table 7. Among the bacterial isolates encountered in the snacks, only *Staphylococcus aureus* did not demonstrate 100% resistance to any of the antibiotics tested. A similar result was reported in 2 out of 3 fungal species isolated from the snacks. They include *Fusarium* sp. and *Aspergillus* sp. All the isolates of *Bacillus* sp. were resistant to cefuroxime. *Escherichia coli* demonstrated a similar result against ceftriaxone, gentamycin, and cefuroxime, while *Pseudomonas* spp. involved nitrofurantoin, ceftazidime and ceftriaxone.

None of the bacterial and fungal isolates from the RTE snacks showed 100% resistance against ofloxacin and erythromycin. Furthermore, 19 out of 39 isolates demonstrated antibiotic resistance against ceftazidime. This is the antibiotic that the lowest number of isolates from the RTE snacks demonstrated resistance against it and recommended for treatment of infections caused by the bacteria. On the contrary, the highest number of isolates demonstrated resistance to nitrofurantoin. Although *Staphylococcus aureus* isolated from the snack samples had the highest percentage occurrence, the isolates did not demonstrate 100% resistance to any of the antibiotics tested. Ngueugang et al. (2021) reported that *Escherichia coli* isolates from RTE foods sold within a primary school demonstrated 100% sensitivity to ciprofloxacin and 100% resistance to erythromycin. All the *Fusarium* and *Penicillium* isolates encountered in the snack samples were sensitive to cefuroxime and ciprofloxacin, respectively. However, all the *Penicillium* isolates were resistant to cefuroxime. The three fungal species isolated from the RTE snacks demonstrated varying levels of resistance to each of the antibiotics tested.

Table 7: Antibiotic Resistance Pattern of Isolates from the RTE Snack

Microbial Isolates	No. of Isolates	NIT (300 µg)	OFL (5 µg)	CAZ (30 µg)	CRO (30 µg)	CN (10 µg)	CXM (30 µg)	E (15 µg)	CIP (5 µg)
Escherichia coli	3	1(33.33)	2 (66.67)	1(33.33)	3 (100.00)	3 (100.00)	3 (100.00)	1 (33.33)	1(33.33)
Enterococcus spp.	5	3 (60.00)	4 (80.00)	2 (40.00)	5 (100.00)	3 (60.00)	5 (100.00)	2 (40.00)	5 (100.00)
Pseudomonas spp.	4	4 (100.00)	3 (75.00)	4 (100.00)	4 (100.00)	2 (50.00)	2 (50.00)	2 (50.00)	3 (75.00)
Staphylococcus aureus	10	8 (80.00)	6 (60.00)	5 (50.00)	7 (70.00)	4 (40.00)	6 (60.00)	7 (70.00)	7 (70.00)
Bacillus spp.	5	3 (60.00)	4 (80.00)	2 (40.00)	4 (80.00)	3 (60.00)	5 (100.00)	3 (60.00)	3 (60.00)
Penicillium spp.	3	2(66.67)	1 (33.33)	1 (33.33)	1 (33.33)	2 (66.67)	3 (100.00)	2 (66.67)	0 (0.00)
Fusarium spp.	4	3 (75.00)	3 (75.00)	2 (50.00)	1 (25.00)	1 (25.00)	0 (0.00)	1 (25.00)	2(50.00)
Aspergillus spp.	5	4 (80.00)	1 (20.00)	2 (40.00)	1 (20.00)	4 (80.00)	2 (40.00)	3 (60.00)	4(80.00)
Total	39	28 (71.79)	24(61.53)	19 (48.72)	26 (66.67)	22 (56.41)	26 (66.67)	24 (61.53)	25 (64.10)

Key: NIT - Nitrofurantoin; OFL - Ofloxacin; CAZ - Ceftazidime; CRO - Ceftriaxone; CN - Gentamycin; CXM - Cefuroxime; E -Erythromycin; CIP - Ciprofloxacin.

CONCLUSION

All the RTE snacks obtained from vendors doing business within the three Primary school premises were contaminated with bacteria and fungal species, with the exception of doughnut sold in ADPS. The dominant microorganism isolated from the RTE snacks was *Staphylococcus aureus*. The snacks obtained from vendors located in ADPS had higher microbiological quality compared with products obtained from other locations. Antibiogram profile obtained from this study demonstrated the efficacy of ceftazidime as a drug of choice for treatment of foodborne illness which could occur after eating the RTE snacks sold within the three primary schools.

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