Research Article

Microbiological quality and antibiotics sensitivity of potential pathogens from roasted groundnuts sold in Rivers State University and its environs

Patience Chisa Obinna-Echem* and Godstime Uchechi Thomas

Food Science and Technology, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria

Abstract

Microbiological quality and antibiotic sensitivity of potential pathogens from roasted skinned and unskinned groundnuts sold in the River State University campus and its environs were evaluated. Samples obtained from three locations namely: Back gate, Main gate and Shopping complex were coded BSG, MSG and SSG respectively for skinned and BUG, MUG and SUG for the unskinned. Samples transported to the laboratory in chilled cooler were analyzed immediately as freshly purchased and after four weeks of cold storage (15.0 ±2°C). Conventional microbiological methods were used for analysis. Microbial analysis (Log\textsubscript{10} CFU/g) revealed that all freshly purchased samples had total bacteria count (TBC) of 3.99 – 5.98, coliform (TCC), Salmonella (TSC) and mould (TMC) counts of 1.00, 4.30 and 5.11 in BUG, SUG and MUG respectively. Escherichia coli (TEC), Staphylococcus (TSTC) and yeast (TYC) counts in three of the samples ranged respectively, from 1.35 – 2.70, 3.00 – 4.30 and 2.00 – 3.70. Total lactobacillus count (TLBC) was 2.70 – 4.49 for MSG and SUG. After storage, the microbial load increased and counts for TBC, TCC, TLBC, TSTC, TEC, TSC, TYC and TMC ranged from 5.04 – 7.89, 3.00 – 4.75, 2.70 – 3.00, 3.78 – 5.23, 1.35 – 4.58, 5.11 in MSG, 1.20 – 4.30 and 2.00 – 3.70. Total lactobacillus count (TLBC) was 2.70 – 4.49 for MSG and SUG. After storage, the microbial load increased and counts for TBC, TCC, TLBC, TSTC, TEC, TSC, TYC and TMC ranged from 5.04 – 7.89, 3.00 – 4.75, 2.70 – 3.00, 3.78 – 5.23, 1.35 – 4.58, 5.11 in MSG, 1.20 – 4.30 and 2.00 – 3.70. Total lactobacillus count (TLBC) was 2.70 – 4.49 for MSG and SUG. After storage, the microbial load increased and counts for TBC, TCC, TLBC, TSTC, TEC, TSC, TYC and TMC ranged from 5.04 – 7.89, 3.00 – 4.75, 2.70 – 3.00, 3.78 – 5.23, 1.35 – 4.58, 5.11 in MSG, 1.20 – 4.30 and 2.00 – 3.70. Total lactobacillus count (TLBC) was 2.70 – 4.49 for MSG and SUG. After storage, the microbial load increased and counts for TBC, TCC, TLBC, TSTC, TEC, TSC, TYC and TMC ranged from 5.04 – 7.89, 3.00 – 4.75, 2.70 – 3.00, 3.78 – 5.23, 1.35 – 4.58, 5.11 in MSG, 1.20 – 4.85 respectively. Antibiotic inhibition against Staphylococcus and Salmonella varied from 1.00 – 14.5 and 4.5 – 14.5 mm. E. coli was sensitive to gentamycin, perfloxacin and ofloxacin with inhibition zones of 8.5, 12.5 and 13.5 mm respectively. Good hygienic practices and appropriate storage facilities will minimize contamination and ensure safe roasted groundnuts for consumption. The sensitivity test indicates what can be utilized in the event of food poison.

1. Introduction

Groundnut (Arachis hypogaea L.), also known as earthnut and peanut is cultivated and consumed in the tropics, sub tropics and temperate regions of the world. The nut is made up of oil and nutrient rich seed having a seed-coat enclosed in an epicarp shell. The seed can be consumed raw, lightly roasted or boiled with or without the shell, processed into a paste that is used as a spread for baked goods, crushed for production of oil, candy bars, cookies and peanut brittle etc. [1]. Nigeria is the largest groundnut producing country in West Africa, accounting for 51% of production in the region [2]. It is an important component of Nigerian diet providing approximately 5% of the estimated 58.9 g of crude protein available per head per day [3]. In Nigeria, especially the southern part, the roasted shelled or unshelled, skinned or unskinned groundnuts in different packing materials such as polyethylene bags, plastic and glass bottles are sold in public places such as markets, offices, schools, motor parks, bus stops,
hospitals, restaurants, supermarkets and also hawked along the expressway in both rural and urban areas. It is also used in entertaining guests in many occasions where it is served with garden eggs as roasted skinned or paste mixed with salt and different spices.

The awareness of nut associated food infection was created by the outbreak of salmonellosis in peanut and peanut products [4, 5]. The preparations in unsanitary environment and packaging materials in addition to poor handling and storage can lead to post processing contamination with microorganisms that predispose to food poisoning. Groundnuts like other nuts are highly susceptible to microbial invasion especially fungal attack at various stages of processing due to low moisture content and rich nutrient content [6]. Due to low water activity, the pathogens may not proliferate but can survive resulting in food poisoning when consumed. Consequently, various microorganisms have been reportedly isolated from groundnuts and their products by different authors especially, bacteria species of the genera Bacillus, Enterobacter, Escherichia, Klebsiella, Shigella, Salmonella, Staphylococcus, Micrococcus, Proteus, Streptococcus and fungi species such as Aspergillus, Penicillium, Mucor Fusarium, Trichoderma and Rhizopus [5, 7-11].

Antibiotics are substances that inhibit the proliferation of bacteria and are used in the body for treatment of various infections. They are of different categories and act in specific ways to achieve inhibition. The fluoroquinolone based antibiotics such as ciprofloxacin, act by inhibiting bacterial DNA gyrase responsible for DNA replication and transportation [12], penicillin and cefuroxime-class such as Amoxicillin, Ampiclox Rocephin and Zinacef and Erythromycin act against the bacterial cell wall synthesis [13]. Gentamicin and streptomycin belong to the aminoglycoside based antibiotics that bind irreversibly to the 16S rRNA subunit of the 30S ribosome and inhibit bacterial protein synthesis [14, 15], some contain substances that are not antibiotics but rather block the enzymes that contribute to antibiotic resistance allowing the antibiotics to function effectively. Example is the Beta-lactamase inhibitors in the clavulanic potassium content of Augmentin [16]. Microbial antibiotic sensitivity test is to find out which antibiotic can inhibit the growth of the pathogen and the best for use in the treatment of any poisoning arising from the consumption of food contaminated with the pathogens.

Some Authors have isolated pathogens in ready to eat groundnuts [5, 7-11]. However, no information on the microbiological quality of groundnuts sold at Rivers State University and its environs. The aim of this study was to evaluate the microbiological quality and antibiotics sensitivity of potential pathogens in ready-to-eat roasted groundnuts sold in Rivers State University and its environs.

2. Materials and methods

2.1 Groundnut samples

Roasted skinned and unskinned groundnut samples were purchased from three locations mainly Main gate, Back gate and Shopping complex of the Rivers State University, Port Harcourt Rivers State, Nigeria. The skilled groundnut samples purchased from the back gate, main gates and shopping complex were coded BSG, MSG and SSG respectively, while the unskinned samples were coded BUG, MUG and SUG. The samples were well labelled and transported in a chilled cooler to the laboratory for analysis. Samples were analyzed immediately after purchases as freshly purchased and after four weeks of cold storage at 15±2°C, mimicking the temperature of display in most supermarkets.

2.2. Microbiological Analysis

Serial dilution to enumeration was carried out as described by Obinna-Echem and Cookey, [17] while the method by Harrigan [18] was followed during isolation and characterization. Antibiotic sensitivity test was as described by Barber et al [19].

2.2.1. Media and plate preparation

Microbial media were prepared and sterilized following the manufacturer’s (Sigma-Aldrich, Burlington, Massachusetts, USA) instruction. Appropriate quantities were weighed into a beaker and dissolved in the right amount of water by gentle heating with constant stirring on a hot plate. Thereafter they were transferred into a 250 mL glass bottle and autoclaved at 121°C for 15 min. The sterile media were cooled to 45°C in a water bath before dispensing approximately 10 mL into sterile Petri dishes to set. Set plates were turned upside down and stored away in the refrigerator till when needed for analysis.
2.2.2. Serial dilution

This was carried out as described by Obinna-Echem and Cook, [17]. Serial dilution for each sample was prepared by homogenizing 10 g of the sample with 90 mL of sterile peptone water. Thereafter, 1 mL was aseptically withdrawn into 9 mL of sterile peptone water in a sterile 20 mL tubes, vortexed for 3-5 s and serially diluted to 10⁵.

2.2.3. Inoculation and incubation

Following the method described by Obinna-Echem and Cook, [17], aliquots (100 µL) of appropriate dilutions: 10⁻⁵ for Nutrient agar (NA), 10⁻² for Eosin methylene blue agar (EMB), Salmonella Shigella agar (SSA), MacConkey agar (MCA) and Mannitol salt agar (MSA), and 10⁻¹ for Potato dextrose agar (PDA), and Sabaroud dextrose agar (SDA) were plated on appropriate microbial media for each microorganism. Total aerobic bacteria, coliform, *Escherichia coli* and *Salmonella* were respectively, enumerated on NA, MCA, EMB and SSA. Total yeast, mould and *Staphylococcus* were enumerated on PDA, SDA, and MSA respectively. Then NA, MCA, and SSA were incubated at 34°C for 24-48 h while PDA and SDA were incubated at 25°C for 48 h and EMB was incubated at 45°C for 48 h.

2.2.4. Enumeration and calculation

After incubation, visible colonies were counted and the microbial numbers calculated as CFU/g = (Number of colonies X Dilution factor)/Volume of inoculum. Values obtained were converted to Log₁₀ CFU/g [17].

2.2.5. Isolation of potential pathogens

Colonies from the selected pathogen media were subcultured by streak plating to obtain pure cultures that were confirmed and used for sensitivity tests. *E. coli* on EMB plates were sub-cultured and incubated at 44°C for 24-48 h, *Staphylococcus* and *Salmonella* were sub-cultured on MSA and SSA respectively and incubated at 37°C for 24-48 h. At the end of the incubation period, plates were examined to ensure that they contained pure cultures. Confirmation of the pure culture was conducted by colony morphology characterization, Gram staining, catalase and coagulase tests as described by Harrigan [18].

2.2.6. Antibiotic Sensitivity test

Antibiotics in the gram-positive disc were: chloramphenicol (30 µg), ampicloix (30 µg), rocephin (30 µg), ciprofloxaxin (10 µg), septrin (30 µg), sparfloxacain (10 µ), erythromycin (30 µg) and pefloxacin (30 µg) while the gram-negative disc had Septrin (30 µg), Ciprofloxacin (10 µg), Amoxicillin (30 µg), Augmentin (30 µg), gentamycin (10 µg), pefloxacin, (30 µg), ofloxacin (10 µg) streptomycin (30 µg), sparfloxacain (10 µg), and chloramphenicol (30 µg). The analysis was carried out as described by Barber et al., [18].

2.3 Statistical Analysis

Data obtained were subjected to statistical analysis using Minitab (Release 18.1) Statistical Software English (Minitab Ltd. Conventry, UK). Statistical differences and relationships among variables were evaluated by analysis of variance under general linear model and Turkey pairwise comparison at 95% confidence level.

3. Results and discussion

Microbial counts of roasted skinned and unskinned groundnuts freshly purchased from different locations in Rivers State University campus and its environs are shown in Table 1, while counts after cold storage are shown in Table 2.

The total bacteria count (TBC) of the roasted skinned and unskinned groundnut samples ranged from 4.30 – 5.98 and 3.99 – 5.98 Log₁₀ CFU/g respectively. After 4 weeks of storage at cold temperature, the counts ranged from 7.19 – 7.89 and 3.98 – 7.06 Log₁₀ CFU/g for the roasted skinned and unskinned groundnut samples respectively. Total Bacteria Count of a substance is a quantitative estimate of the number of microorganisms present in a sample. The result revealed that there was no significant difference (p>0.05) between the TBC of skinned and unskinned roasted groundnut samples but there was significant (p<0.05) increase in TBC after storage. The result of the freshly purchased samples were comparable to the findings made by Akinnibosun and Osawaru [20] that TBC in unskinned groundnut sold in Benin City in the range of 0.5 - 2.1 x 10⁴ CFU/g equivalent to 3.70 – 4.32 Log₁₀ CFU/g and Oranusi and Braide [21] that TBC in groundnut sold along Onitsha-Owerri expressway in the range of 1.1 - 58.0 x 10⁴ CFU/g equivalent to 4.54 – 5.76 Log₁₀ CFU/g. TBC is also known as total viable or aerobic count and it is the total number of bacteria able to grow in an aerobic environment in moderate temperature. There are no applicable limits in ready
to eat foods [22]. However, high levels indicate general poor quality and reduction in shelf life due to storage and handling problems. The roasted groundnuts are packaged in open environment with the possibility of contaminants in the environment settling on them in addition to the hands of the food handlers.

The result of total coliform count (TCC) indicated that there was no coliform growth in all the freshly purchased samples except for unskinned sample from the back gate (BUG) that had a count of 1.00 Log_{10} CFU/g. After storage, there was significant (p<0.05) growth of coliform in the samples, the values ranged from 3.70 – 4.75 and 3.00 – 3.20 Log_{10} CFU/g respectively, for the skinned and unskinned samples. These values are in line with the report of 3.5 x 10^2 – 4.3 x 10^4 CFU/g equivalent to 2.54 – 4.63 Log_{10} CFU/g for groundnut sold along Onitsha-Owerri expressway [21]. This may imply that the groundnuts sold on the express must have either been stored for some time or the conditions on the highways had made increased contamination or growth compared to the freshly purchased samples. Coliform is a group of microorganisms in the Enterobacteriacaea family and the value obtained in this study was within the borderline of 2 – 4 Log_{10} CFU/g given by Centre for Food Safety, [22] with the exception BSG and MSG after storage.

Total lactobacillus count (TLBC) in the freshly purchased roasted groundnut samples, was only observed in sample MSG with count of 2.70 Log_{10} CFU/g for the roasted skinned groundnut and sample SUG with count of 4.49 Log_{10} CFU/g for the unskinned groundnut samples. After storage, the TLBC in the skinned sample ranged from 1.35 – 4.48 Log_{10} CFU/g in samples BSG and MSG, respectively while the

<table>
<thead>
<tr>
<th>Samples</th>
<th>TBC</th>
<th>TCC</th>
<th>TEC</th>
<th>TSTC</th>
<th>TLBC</th>
<th>TSC</th>
<th>TYC</th>
<th>TMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSG</td>
<td>5.20±0.71</td>
<td>NG</td>
<td>1.35±1.91</td>
<td>NG</td>
<td>NG</td>
<td>2.00±0.00</td>
<td>NG</td>
<td></td>
</tr>
<tr>
<td>BUG</td>
<td>5.98±0.00</td>
<td>1.00±0.00</td>
<td>2.70±0.00</td>
<td>3.60±0.43</td>
<td>NG</td>
<td>NG</td>
<td>3.70±0.00</td>
<td>NG</td>
</tr>
<tr>
<td>MSG</td>
<td>5.98±0.03</td>
<td>NG</td>
<td>3.00±0.01</td>
<td>2.70±0.01</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>MUG</td>
<td>3.99±0.35</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>2.70±0.01</td>
<td>NG</td>
</tr>
<tr>
<td>SUG</td>
<td>4.30±0.00</td>
<td>4.30±0.00</td>
<td>2.70±0.00</td>
<td>3.70±0.00</td>
<td>NG</td>
<td>NG</td>
<td>2.70±0.01</td>
<td>NG</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation of duplicate samples. Means within a column with different superscripts are significantly different at (p<0.05). BSG = Back gate skinned roasted groundnut, BUG = Back gate unskinned roasted groundnut, MSG = Main gate skinned roasted groundnut, MUG =Main gate unskinned roasted groundnut, SSG = Shopping complex skinned roasted groundnut, SUG = Shopping complex unskinned roasted groundnut, NG = No growth, TBC =Total bacteria count, TCC = Total coliform count, TEC = Total Escherichia coli count, TSTC = Total Staphylococcus count, TLBC =Total Lactobacillus count, TSC = Total Salmonella Count, TYC = Total yeast count, TMC = Total mould count.

<table>
<thead>
<tr>
<th>Samples</th>
<th>TBC</th>
<th>TCC</th>
<th>TEC</th>
<th>TSTC</th>
<th>TLBC</th>
<th>TSC</th>
<th>TYC</th>
<th>TMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSG</td>
<td>7.89±0.07</td>
<td>4.75±0.01</td>
<td>2.70±0.00</td>
<td>4.84±0.01</td>
<td>1.35±1.91</td>
<td>NG</td>
<td>1.20±1.70</td>
<td>4.74±0.00</td>
</tr>
<tr>
<td>BUG</td>
<td>7.06±2.21</td>
<td>3.00±0.00</td>
<td>NG</td>
<td>3.95±0.00</td>
<td>NG</td>
<td>NG</td>
<td>2.70±0.00</td>
<td>2.70±0.00</td>
</tr>
<tr>
<td>MSG</td>
<td>7.37±0.59</td>
<td>4.65±0.00</td>
<td>2.70±0.00</td>
<td>4.90±0.00</td>
<td>4.48±0.00</td>
<td>5.11±0.00</td>
<td>4.85±0.00</td>
<td>2.70±0.00</td>
</tr>
<tr>
<td>MUG</td>
<td>3.98±0.00</td>
<td>3.20±0.71</td>
<td>NG</td>
<td>3.98±0.00</td>
<td>3.00±0.00</td>
<td>NG</td>
<td>2.70±0.00</td>
<td>2.70±0.00</td>
</tr>
<tr>
<td>SSG</td>
<td>7.19±0.55</td>
<td>3.70±0.00</td>
<td>NG</td>
<td>5.23±0.00</td>
<td>NG</td>
<td>NG</td>
<td>4.19±0.41</td>
<td>3.94±0.34</td>
</tr>
<tr>
<td>SUG</td>
<td>5.04±0.00</td>
<td>3.00±0.00</td>
<td>NG</td>
<td>3.78±0.00</td>
<td>NG</td>
<td>NG</td>
<td>2.70±0.00</td>
<td>2.70±0.00</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation of duplicate samples. Means within a column with different superscripts are significantly different at (p<0.05). BSG = Back gate skinned roasted groundnut, BUG = Back gate unskinned roasted groundnut, MSG = Main gate skinned roasted groundnut, MUG =Main gate unskinned roasted groundnut, SSG = Shopping complex skinned roasted groundnut, SUG = Shopping complex unskinned roasted groundnut, NG = No growth, TBC =Total bacteria count, TCC = Total coliform count, TEC = Total Escherichia coli count, TSTC = Total Staphylococcus count, TLBC =Total Lactobacillus count, TSC = Total Salmonella Count, TYC = Total yeast count, TMC = Total mould count.
unskinned sample had count of 3.00 Log\(^{10}\) CFU/g for sample MUG. The low count of Lactobacillus spp a fermentative organism in the groundnut samples was satisfactory since it is not a fermented product. However, this observation may not be unconnected with the ubiquitous nature and can come from the environment, food handlers, packaging materials etc. These were not of any quality or safety issue in the dried ready to eat nuts.

The total *E. coli* counts (TEC) in the freshly purchased samples were 1.35, 2.70 and 2.70 Log\(^{10}\) CFU/g for BSG, SSG and BUG respectively. After storage, there was significant (p<0.05) increase in the TEC of BSG and BUG with values of 2.70 and 3.00 Log\(^{10}\) CFU/g respectively, while there was no increase in SSG. The presence of *E. coli* in the samples could be due to the hygienic condition of the area as well as the extent of hygienic practices by the vendors since *E. coli* is a common faecal indicator organism. Its presence in food generally indicates direct or indirect faecal contamination [22]. According to the guidelines for ready to eat foods by CFS, [22] the satisfactory level for *E. coli* is <20 CFU/g (equivalent to <1.03 Log CFU/g) while >10\(^{3}\) CFU/g (equivalent to >2 Log CFU/g) is unsatisfactory. The level of *E. coli* detected in sample BSG, SSG and BUG before and after storage are unsatisfactory and adequate personal hygiene practices, clean environment and use of clean packing materials are highly solicited. Though in developed world, this would call for investigation of the vendors.

The total *Staphylococcus* count (TSTC) showed that the freshly purchased sample MSG and SSG had counts of 3.00 and 4.30 Log\(^{10}\) CFU/g respectively, while there was no growth in BSG. In the unskinned groundnut samples, there was no growth in sample MSG and BSG while BUG had counts of 3.60 Log\(^{10}\) CFU/g. After storage, the skinned samples had TSTC in the range of 4.84 – 5.23 Log\(^{10}\) CFU/g for BSG and SSG respectively. The unskinned samples had counts of 3.78 -3.98 Log\(^{10}\) CFU/g respectively for SUG and MUG. The presence of *Staphylococcus* in the ready to eat roasted skinned and unskinned groundnuts is in accordance with the finding of Kigigha et al., [10]. The values were higher than 1.92 – 2.29 Log\(^{10}\) CFU/g reported for unpeeled groundnut sold in some locations in Yenagoa metropolis, Bayelsa state, Nigeria [10]. The levels in the freshly purchased samples except for SSG and all the unskinned samples after storage were within the satisfactory limits of <4 Log\(^{10}\) CFU/g [22]. Consumption of groundnut with unsatisfactory levels is a potential risk to health although for the production of the heat-stable toxin levels >5 Log\(^{10}\) CFU/g is required.

The total *Salmonella* count (TSC) revealed that the freshly purchased samples, had no growth of *Salmonella* except for SUG with count of 4.30 Log\(^{10}\) CFU/g. After storage, there was increase in growth of *Salmonella* in SUG to 5.11 Log\(^{10}\) CFU/g. The total *Salmonella* count in this study has similarity with the study carried out by Kikigha et al., [10], where *Salmonella shigella* was not detected in the various groundnut samples. The approved safety level for *Salmonella* in ready to eat nut like groundnut is absence of the pathogen (No growth) [22]. Sample SUG with *Salmonella* growth is unfit for consumption, though this might be difficult to avoid as food with pathogens may look good as with the groundnut samples.

The total yeast count (TYC) in the freshly purchased roasted skinned groundnut samples was 2.00 – 2.70 Log\(^{10}\) CFU/g for samples from BSG and SSG, respectively. There was no growth in the unskinned samples except for sample BUG with the count of 3.70 Log\(^{10}\) CFU/g. The freshly purchased samples had no mold growth except for sample MUG with count of 2.7 Log\(^{10}\) CFU/g. After storage, the total mould count of the roasted skinned groundnuts ranged from 2.70 – 4.74 Log\(^{10}\) CFU/g for samples MSG and BSG, respectively, while the roasted unskinned groundnuts had count of 2.70 Log\(^{10}\) CFU/g for all the samples. The fungi result from this present study is in agreement with other reports on groundnut and its products sold in other locations in Nigeria [8, 10, 20, 21]. The major challenge with the presence of fungi in groundnuts is the production of mycotoxins. Some fungi diversity found in groundnuts revealed toxin producing microbes such as species of *Penicillium, Fusarium* and *Aspergillus* that are known to produces mycotoxins in food [13, 23].

The characteristics of isolated potential pathogens are shown in Table 3. The preliminary identification showed that the pathogens are *Staphylococcus aureus, Escherichia coli* and *Salmonella* Spp. Fig. 1 showed the antibiotic sensitivity of the isolated *Staph. aureus* on a gram-positive disc while the antibiotic sensitivity
Table 3. Characteristics of isolated potential pathogens from roasted skinned and unskinned groundnut from Rivers State University and its environs.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Colony on media</th>
<th>Morphological characteristics</th>
<th>Biochemical test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Shape</td>
<td>Gram Reaction</td>
</tr>
<tr>
<td>Salmonella</td>
<td>Opaque, round</td>
<td>Short</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>large on SSA</td>
<td>Scattered in pairs and some</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>single</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>Greenish to black</td>
<td>Short</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>on EMB</td>
<td>Scattered singly, in pairs</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>and small</td>
<td></td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>Smooth, thick</td>
<td>cocci</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>yellow to orange</td>
<td>clusters</td>
<td></td>
</tr>
<tr>
<td></td>
<td>on MSA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

of the isolated E. coli and Salmonella on a gram-negative discs are shown in Fig. 2. The antibiotic sensitivity test evaluated potential antibiotic that can inhibit the growth of the pathogen for effective treatment of any poisoning arising from the consumption of the roasted groundnuts.

![Figure 1](image1.png)

Figure 1. Antibiotic inhibition zones against Staphylococcus isolated from roasted skinned and unskinned groundnut from Rivers State University and its environs. (Bars and error bars represent mean inhibition zones and standard deviation of duplicate measurement. Bar with the same superscript are not significantly different at p<0.05. BUG – Back gate roasted unskinned groundnut, MSG - Main gate roasted skinned groundnut, SSG – Shopping complex roasted skinned groundnut)

The antibiotics used from the gram-positive disc were chloramphenicol, ampiclox, rocephin, ciprofloxacin, septrin, sparflloxacin, erythromycin and pefloxacin. Staphylococcus spp isolated from roasted skinned groundnut sample from back gate (BSG) was resistant to all the antibiotics except for pefloxacin and ciprofloxacin with inhibition zones of 1.00 and 9.50 mm respectively. Isolate from skinned roasted groundnut from Shopping complex (SSG) was resistant to gentamycin and amoxicillin but had inhibition zones in the range of 11.5 – 14.5 for erythromycin and ciprofloxacin respectively. Ciprofloxacin was inhibitory to all the Staphylococcus spp isolated and had significantly (p<0.05) the highest inhibition zones.

![Figure 2](image2.png)

Figure 2. Antibiotic inhibition zones against Salmonella and E. coli isolated from roasted skinned and unskinned groundnut from Rivers State University and its environs. (Bars and error bars represent mean inhibition zones and standard deviation of duplicate measurement. Bar with the same superscript are not significantly different at p<0.05. MUG - Main gate roasted unskinned groundnut, BUG – Back gate roasted unskinned groundnut)

The antibiotics from the gram-negative disc were septrin, ciprofloxacin, amoxicillin, augmentin, gentamycin, pefloxacin, ofloxacin, streptomycin, sparflloxacin, and chloramphenicol. Salmonella was resistant to chloramphenicol and sensitive to all the other antibiotics with inhibition zones in the range of 4.5–14.5 mm for septrin and ciprofloxacin respectively.
The inhibitory zones of sparﬂoxacin, augmentin and ofloxacin against Salmonella did not differ significantly (p>0.05) from ciprofloxacin. E. coli was sensitive to gentamycin, pefloxacin and ofloxacin with inhibition zones of 8.5, 12.5 and 13.5 mm respectively.

The inhibitory activities of antibiotics involve interference with cell wall synthesis, strength and rigidity; DNA replication and protein synthesis; and blockage of enzymes that increases resistance in pathogens [12, 15-16, 24]. The sensitivity of the pathogens was signiﬁcantly (p<0.05) higher with the ﬂuoroquinolone antibiotics: ciprofloxacin, sparﬂoxacin, ofloxacin and pefloxacin that act by inhibiting DNA replication. This is in line with the report by Barber et al., [19] and conﬁrmed the report of excellent activities against gram-negative and gram-positive bacteria [25]. The resistance of gram-negative bacteria to antibiotics is usually attributed to the induction, mutation or by acquisition of R-plasmids, or the inability of the antibiotics to reach the active site [19]. The sensitivity of these potential pathogens to the different antibiotics implies that in the event of food poisoning from the consumption of contaminated roasted groundnut, the use of such antibiotics can help in alleviating the situation. Only selected antibiotics can be used for the Staphylococcus isolated from roasted groundnut from the main gate and shopping complex while for isolate from back gate only ciprofloxacin and pefloxacin will be helpful. The isolated Salmonella can be handled with a wide range of antibiotics while gentamycin, pefloxacin and ofloxacin will be effective for the isolated E coli.

4. Conclusions
The study revealed that all the freshly purchased samples had total bacteria count; coliform, Salmonella and mould were found in one sample: BUG, SUG and MUG respectively, three of the samples had E. coli, Staphylococcus and yeast counts and two samples (MSG and SUG) had lactobacillus. After storage, the microbial counts revealed high level of total bacteria count (TBC), borderline level of coliform (TCC), safe level of lactobacillus (TLBC), satisfactory level of Staphylococcus (TSTC), unsatisfactory level of E. coli (TEC), unsafe level of Salmonella (TSC) and high level yeast (TYC) and mould (TMC). Proper processing, hygienic practices and good storage facilities will minimize contamination and ensure safe roasted groundnuts for consumption. Isolated pathogens were Staphylococcus aureus, Escherichia coli and Salmonella. They had varying sensitivities to antibiotics. Inhibition of the pathogens by selected antibiotics suggests the likely antibiotics that can be utilized in checking their proliferation and ill effect on consumers.

Authors’ contributions
Concept, data analysis, literature search, final draft, and supervision, O.E.P.C.; Sample collection and laboratory analyses, T.G.U.

Acknowledgements
The authors appreciate the technical assistance of Ms. Earnest Nwiidebom of the Microbiological Laboratory at the Department of Food Science and Technology.

Funding
The authors received no external funding.

Availability of data and materials
All data will be made available on request according to the journal policy.

Conflicts of interest
Authors have declared that no competing interests exist.

References


22. CFS. Centre for Food Safety. Microbiological Guidelines for Food (For ready-to-eat food in general and specific food items). Food and Environmental Hygiene Department 43/F, Queensway Government Offices, 66 Queensway, Hong Kong. August 2014 (revised)

