Antibiogram Testing of Bacterial Isolates from Cassava, Yam and Plantain Flours and Shelf Life Studies of the Products Sold in Some Markets in Port Harcourt, Nigeria

M. Elenwo¹, N. Maduka²* and N. N. Odu¹

¹Department of Microbiology, Faculty of Science, University of Port Harcourt, Port Harcourt, Nigeria.
²Department of Microbiology, College of Natural and Applied Sciences, Wellspring University, Benin City, Edo State, Nigeria.

Authors’ contributions

This work was carried out in collaboration among all authors. Author NNO designed and supervised the study. Author NM performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author ME managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Bacterial resistance to commonly used antibiotics is a threat to public health. This study focused on antibiogram testing of bacterial isolates from packaged and exposed cassava, plantain and yam flour using chloramphenicol, ciprofloxacin, erythromycin, levofloxacin, gentamicin, ampiclox, rifampicin, amoxil, streptomycin, norfloxacin, ampicillin, cephorex, tarivid, nalidixic acid, peflacine, augmentin and septrin. Shelf life studies involved monitoring total viable count, total fungal count and moisture content of the flour samples stored at room temperature (28± 2°C) at 1 Wk interval for 4 Wks. Standard methods and agar diffusion technique were adopted. Bacillus sp. and Staphylococcus sp. (Gram-positive) were identified in all the flour samples. Salmonella sp. and Escherichia coli (Gram negative) were isolated from exposed plantain and yam flour, respectively.

*Corresponding author: Email: maduks.mn@gmail.com;
Gram-positive isolates susceptible and resistant to each antibiotic ranged between 65.38-96.15% and 3.85-30.77%, respectively whereas that of Gram-negative isolates was between 25-75%. Antibiogram testing revealed that 76.92 and 30.77% of *Bacillus* and *Staphylococci* isolates, respectively were sensitive; 100% *Salmonella* sp. and *E. coli* (isolate OMY) was resistant and sensitive, respectively. Among antibiotics used for Gram-positive bacteria, rifampicin was most effective whereas streptomycin, tetracycline, nalidixic acid, gentamycin, augmentin and ciprofloxacin were more effective than other antibiotics used for Gram negative bacteria. During storage of the flour samples, moisture content (8.6-23.20%) and total viable count (6.47-23.20 log<sub>10</sub> cfu/g) increased but total fungal count (3.53-2.15 log<sub>10</sub> cfu/g) decreased with few exceptions. Therefore, reduction in microbial contamination of edible flours by implementing good manufacturing practices and proper packaging of the commercialized products could reduce the spread of antimicrobial resistant bacteria.

**Keywords:** Antibiogram; shelf life; bacterial isolates; flours.

1. **INTRODUCTION**

Cassava, plantain and yam flour is a high starchy meal popular among rural and urban dwellers in southern Nigeria [1]. These food powders are increasingly patronized by millions of individuals [2]. Generally, powdered starchy staples are regarded as microbiologically safe because of its low water activity. However, studies have revealed that during the traditional processing of flour, pathogenic and non-pathogenic microorganisms usually contaminate the product [3]. Furthermore, post-processing steps and improper storage condition of flour from starchy staples could encourage the growth of several microorganisms implicated in food spoilage [4,5,6].

Pre-harvesting and post-harvesting factors predispose food to microbial contamination [7]. The shelf life of edible flour and safety of the food product could be affected by storage conditions and type of packaging material used [8]. The shelf life of exposed edible flours available in open markets in Nigeria is usually not specified by the producers and chance of product contamination is most likely to be higher compared with that of packaged edible flours. Uncontrolled activities of spoilage and pathogenic microorganisms in any food product such as flour meals of yam, cassava or plantain could impact the shelf life and safeness of the product. Consumption of microbial contaminated edible flour is likely to cause human illnesses. Foodborne outbreaks could be traced to the consumption of contaminated edible flour [1]. Several illnesses especially diarrhea and typhoid fever is often associated with consumption of contaminated foods. This constitutes a health burden in many developing countries [1,9]. Typhoid, cholera, *E. coli* gastroenteritis, campylobacteriosis, paratyphoid fever, amoebiasis and poliomyelitis and development of cancer has also been linked to the consumption of contaminated foods [7].

Antibiotics are used in the treatment of illness caused by pathogenic bacteria. Antibiotic resistant bacteria which contaminate food are easily transmitted to humans. This could cause treatment failures which could be life-threatening [10]. Pathogenic bacteria resistance to commonly used antibiotics is a challenge to public health. In fact, antimicrobial resistance is posing a serious challenge to food safety [11,12]. Aruwa and Ogundare [9] investigated the susceptibility of bacteria isolated from fermented cassava flour and plantain flour to several antibiotics. The Gram positive bacterial isolates from the flour samples subjected to antibiotic susceptibility testing revealed that pefloxacin demonstrated the widest zone of inhibition (22.3 mm) and least zone of inhibition (7 mm) against *Corynebacterium* sp. and *Bacillus subtilis*, respectively [9]. Susceptibility testing of selected antibiotics against Gram-negative isolates from the flour samples also revealed that ciprofloxacin demonstrated the least zone of inhibition (11 mm) against *Klebsiella* sp. while ofloxacin recorded widest zone of inhibition (29 mm) against *Enterobacter* sp. [9].

Since frequent monitoring of spread of antibiotic-resistant food pathogens is important and short shelf life of microbial contaminated edible flours could pose a challenge to public health, this study is aimed at carrying out antibiogram testing of bacteria isolated from packaged and exposed yam, cassava and plantain flour as well as shelf life study of the products placed in some markets in Port Harcourt metropolis, Rivers State.
2. MATERIALS AND METHODS

Thirty (30) samples (15 from open markets and 15 from supermarkets) of yam, cassava, and plantain flours were used for this study. Packaged flour samples were obtained from Everyday, Sugarland and Spar supermarkets while exposed flour samples were purchased from Rumuokoro, Mile 3 and Oil mill markets, all in Port Harcourt metropolis. The pre-packaged ground products were purchased as packaged by the producer whereas exposed ground products (1 kg each) were purchased from market sellers using a sterile container. All the flour samples were transported under ambient temperature (28±2°C) to Food and Industrial Microbiology laboratory for analysis.

2.1 Microbiological Analysis

Total viable count and total fungal count in packaged and exposed cassava, yam and plantain flour were determined using the methods described by Odu et al. [13], Eman and Sarifar [14]. Determination of Salmonella sp. in the flour samples involved APHA method [15]. Identification of the bacterial isolates was done using methods described by Cheesbrough [16].

2.2 Antibiotic Susceptibility Assay

Susceptibility of bacterial isolates to selected antibiotics was carried out using agar diffusion technique (Kirby-Bauer NCCLS modified disc diffusion technique) on Mueller-Hinton agar as described by Tagoe et al. [17] and Odu and Afolami [18]. Eighteen-hour old bacteria culture were plated and counter-reacted by antibiotics-seeded multidisc (Oxoid-UK). Chloramphenicol (CH) 30 µg, ciprofloxacin (AU) 10 µg, erythromycin (E) 30 µg, levofloxacin (LEV) 20 µg, gentamycin (CN) 10 µg, ampiclox (APX) 20 µg, rifampicin (RD) 20 µg, amoxil (AML) 20 µg, streptomycin (CN) 30 µg and norfloxacin were used for Gram positive bacteria while streptomycin (CN) 30 µg, ampicilllin (PN) 30 µg, ceporex (CEP) 10 µg, tarivid (OFX) 10 µg, nalidixic acid (NA) 30 µg, peflacin (REF) 10 µg, gentamycin (CN) 10 µg, augmentin (CPX) 30 µg, ciprofloxacin (AU) 10 µg and septrin (SXT) 30 µg were used for Gram negative bacteria. Susceptibility pattern was determined based on clear zone of inhibition observed surrounding any of the bacterial isolates on the plates. The clear zones were measured using a ruler and results expressed in millimeter. Mean zone of inhibition for each bacterial isolates in triplicates were grouped into susceptible (≥ 17 mm), intermediate (13-17 mm), and resistant (0-12 mm).

2.3 Shelf Life Studies

Exposed and packaged cassava, yam and plantain flour samples were stored on a shelf inside Food and Industrial Microbiology Laboratory, the University of Port Harcourt for 4 Wks. Total viable count (TVC) and total fungal count (TFC) of the flour samples were monitored at 1 Wk interval using standard microbiological methods described by Odu et al. [13]; Eman and Sarifar [14]. The bacterial isolates were identified using the method described by Cheesbrough [16]. The moisture content of the flour samples was also monitored at 1 Wk interval for 4 Wks using the method described by Bradely [19].

2.4 Statistical Analysis

Mean values of each parameter analyzed were determined and standard errors calculated.

3. RESULTS

Table 1 shows that *Bacillus* and *Staphylococcus* sp were isolated from exposed and packaged cassava, yam and plantain flour. In addition to these two bacterial genera, *Escherichia coli* and *Salmonella* sp. were also isolated from exposed yam and plantain flour, respectively.

Result presented in Table 2 depict that *Bacillus* sp were sensitive to all the antibiotics tested except isolates EDCPI, OMY and EDYP2 that were resistant to norfloxacin and EDCP1 resistant to streptomycin. All *Staphylococcal* isolates showed resistance to at least one antibiotic except isolate OMC, MTC2, MTP2 and SRCP1. Remarkably, none of the *Staphylococci* and *Bacillus* isolates was resistant to rifampicin. Table 3 shows that *Escherichia coli* (isolate OMY) was sensitive to all antibiotics tested whereas isolate ROY1 and ROY2 showed susceptibility, resistance and intermediate resistance to the various antibiotics. A striking observation in Table 3 is that *Salmonella* sp. isolated from exposed plantain flour was resistant to all the antibiotics tested.

Fig. 1 shows that 96.15% of Gram-positive bacterial isolates (*Bacillus* and *Staphylococci* spp.) were sensitive to chloramphenicol, erythromycin and streptomycin. Also, 30.77% of the isolates were resistant to norfloxacin. Each antibiotic showed intermediate susceptibility to at
least one isolate except streptomycin, erythromycin, chloramphenicol, levofloxacin, gentamicin and amoxicillin. Fig. 2 shows that 75% of Gram negative bacterial isolates (E. coli and Salmonella sp.) were resistant to ceporex, peflacin and septrin but sensitive to streptomycin, tarivid, nalidixic acid, gentamycin, augmentin and ciprofloxacin but sensitive to ampicillin, ceporex, peflacine and septrin. Fifty percentage of bacterial isolates demonstrated intermediate sensitivity to amoxicillin.

Shelf life studies depicted in Figs. 3 and 4 show that product moisture increased steadily during storage. The moisture content of exposed

Table 1. Bacteria isolated from edible flour samples obtained from open markets and supermarkets

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>Edible flour sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus sp.</td>
<td>Packaged and exposed cassava, plantain and yam flour</td>
</tr>
<tr>
<td>Staphylococcus sp.</td>
<td>Packaged and exposed cassava, plantain and yam flour</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Exposed yam flour</td>
</tr>
<tr>
<td>Salmonella sp.</td>
<td>Exposed plantain flour</td>
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Table 2. Antibiogram test for gram positive bacterial isolates

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<th>Levofloxacin</th>
<th>Gentamicin</th>
<th>Ampiclox</th>
<th>Rifampicin</th>
<th>Amoxil</th>
<th>Streptomycin</th>
<th>Norfloxacin</th>
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<td>Bacillus sp</td>
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<td>Staphylococcus sp</td>
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Key: C - Exposed Cassava Flour; CP - Packaged Cassava Flour; P - Exposed Plantain Flour; PP - Packaged Plantain Flour; Y - Exposed Yam Flour; YP - Packaged Yam Flour; S-Sensitive; I-Intermediate; R-Resistance
cassava, plantain and yam flour ranged between 13.3-19.4%, 15.3-23.2% and 8.6-17.0%, while that of packaged flour samples is 10.3-18.2, 13.3-19.5 and 9.6-15.1%, respectively. Similarly, total viable count of exposed cassava, plantain and yam flour ranged between 6.49-6.85, 6.69-6.86 and 6.49-6.60 log$_{10}$cfu/g, while that of packaged flour samples was 6.47-6.78, 6.47-6.78 and 6.47-6.60 log$_{10}$cfu/g, respectively. However, fungal count in the stored flour samples decreased within the storage period with few exceptions between Wk 3-4. Fungal count of exposed cassava, plantain and yam flour ranged between 3.47-2.78, 3.53-2.78 and 3.54-2.85 log$_{10}$cfu/g, while that of packaged flour samples was 3.47-2.60, 3.47-2.68 and 3.53-2.78 log$_{10}$cfu/g, respectively.

4. DISCUSSION

It is a potential hazard for Staphylococcus aureus, Bacillus sp. and Salmonella sp. to be present in any food product because these pathogens are responsible for food-borne diseases [20,21]. This study has shown that S. aureus and Bacillus sp. were present in exposed and packaged cassava, plantain and yam flour. This result is in agreement with findings from a related study by Agwa and Ossai-Chidi [22]. In addition to these two bacteria genera, Salmonella sp. and Escherichia coli were also isolated from exposed yam flour. Isolation of S. aureus and Escherichia coli from the flour samples is a pointer that these products were subjected to unhygienic practices, too much personnel handling, use of poor quality water during processing and undue exposure during retailing [4]. Since Salmonella sp. and S. aureus has the ability to tolerate low water activity, this could also have contributed in their survival in exposed plantain flour and other flour samples (exposed and packaged) generally considered as being microbiologically safe [23,24].

In a related study that involved assessment of the microbiological quality of maize flour sold in major markets in Benin City, Nigeria, Imariagbe et al. [5] reported the presence of Bacillus sp. which recorded 41.6% frequency of occurrence. Studies by Somorin et al. [3] reported that Bacillus megaterium and Staphylococcus saprophyticus were present in yam flour samples milled in the market. Another related study by Gachere et al. [4] reported the presence of Staphylococcus aureus and coliforms in cassava flour obtained from markets in Nairobi Mombasa. Findings from a related study carried out by Aruwa and Ogundare [9] showed that Bacillus sp. was present in plantain flour sold in Urban market in Akure, Ondo State, Nigeria. Staphylococcus aureus isolated from exposed and packaged cassava, plantain and yam flour could have resulted from nasal canals and infected hands of persons handling the products. Studies have demonstrated that S. aureus is capable of producing diverse staphylococcal enterotoxins (SEs) linked to the manifestation of staphylococcal food poisoning (SFP) [23,24]. Staphylococcus aureus is the causative agent for staphylococcal food-borne disease (FBD) which has been linked with FBD outbreaks. Such disease outbreaks impose huge economic burden to the population affected. In another related study, Ajayi [25] isolated E. coli, Bacillus cereus, B. globisporus and B. circulans from dry plantain flour.

Isolation of Salmonella sp. only from exposed plantain flour could be as a result of edible flour generally is not rich in nutrients that favour the growth of Salmonella sp. compared with eggs, meat and egg products where Salmonella sp. is highly prevalent. Further studies have also reported the presence of Salmonella sp. in spices, cheese, fish, ice cream and cake [5,12]. Nontyphoidal Salmonella sp. has been identified as one of the major cause of food-borne

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**Table 3. Antiibiogram test for gram negative bacterial isolates**

<table>
<thead>
<tr>
<th>Isolate code</th>
<th>Streptomycin</th>
<th>Ampicillin</th>
<th>Ceftazidime</th>
<th>Tetracycline</th>
<th>Nalidixic acid</th>
<th>Gentamicin</th>
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<td>Salmonella sp.</td>
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Fig. 1. Susceptibility pattern of Gram positive bacterial isolates to antibiotics

Fig. 2. Susceptibility pattern of Gram negative bacterial isolates to antibiotics
Fig. 3. Total fungal count in edible flours stored at room temperature (28±2°C)
Key: C-Exposed cassava flour; CP-Packaged cassava flour; P - Exposed plantain flour; PP - Packaged plantain flour; Y - Exposed yam flour; YP - Packaged yam flour

Fig. 4. Total bacterial count in edible flours stored at room temperature (28±2°C)
Key: C-Exposed cassava flour; CP-Packaged cassava flour; P - Exposed plantain flour; PP - Packaged plantain flour; Y - Exposed yam flour; YP - Packaged yam flour

Fig. 5. Moisture content of edible flours stored at room temperature (28±2°C)
Key: C-Exposed cassava flour; CP-Packaged cassava flour; P - Exposed plantain flour; PP - Packaged plantain flour; Y - Exposed yam flour; YP - Packaged yam flour
diseases in some European countries [23]. Although occurrence of *Salmonella* sp. in the flour samples was only in exposed plantain flour, it remains a threat to public health because this bacterium can cause disease in humans at very low doses. This is the reason food manufacturers consider the prevalence of *Salmonella* sp. in raw ingredients and its ability to survive for some months in dry foods that have low water activity such as edible flour as a major biological hazard [24]. A related study by Omohimi et al. [6] reported the absence of *Salmonella* sp. in yam flour. This result supports the findings of this study. Meanwhile, the presence of *Escherichia coli* in yam flour reported by Omohimi et al. [6] is in agreement with the result of this study.

Occurrence of *Escherichia coli* in exposed yam flour could be attributed to exposure to the atmosphere, poor hygienic conditions and faecal contamination. In a related study, Somorin et al. [3] reported that *E. coli* and some other microorganisms contaminated white yam flour. According to their report, likely source of contamination was milling machine used in processing yam tubers into flour as well as the method of milling. The presence of *E. coli* and *Salmonella* sp. only in exposed yam flour, as well as *Bacillus* sp. and *Staphylococcus* sp., could be the reason food poisoning resulting from consumption of yam flour was earlier reported by some families in Nigeria which recorded high severity in children. This report is worrisome because the outbreak of food poisoning linked with contaminated yam flour meals is on the increase in Nigeria [3].

Ciprofloxacin is regarded as a broad spectrum antibiotic. It is more sensitive to Gram negative than Gram-positive bacteria. This study shows that all the *Bacillus* isolates were sensitive to ciprofloxacin. Antibiotics susceptibility assay of *Escherichia coli*, *S. aureus* and *Salmonella Typhi* against ciprofloxacin reported by Ali et al. [26] shows some level of similarity with the findings from this study. A related study by Oluyeye et al. [27] which involved antibiotic resistance profile of bacterial isolates from ready-to-eat indigenous foods such as pounded yam reported that *E. coli* which represent 2 (8.70%) of the bacterial isolates showed resistance to nalidixic acid; all the *Salmonella* isolates were resistant to gentamicin; 4 (40%) were resistant to nalidixic acid and 10 (100%) were resistance to augmentin is similar to the results from this study. Results from this study show that all the *Bacillus* isolates except EDCP1 were sensitive to gentamycin and streptomycin. This result is in agreement with findings by Aruwa and Ogundare [9] from a related study. They reported that *B. subtilis* was resistant to amoxil, ciprofloxacin, erythromycin and ampiclox. Our results shows that all the *Bacillus* and *Staphylococcus* isolates except SRYP2 were sensitive to chloramphenicol. In a related study, Imaeriagbe et al. [5] reported that bacterial isolates from maize flour were also sensitive to chloramphenicol.

Antibiogram testing shows that *Salmonella* sp. isolated only from exposed yam flour was resistant to all the antibiotics used in this study. The *Salmonella* sp. could be multidrug-resistant (MDR) strain which is relatively ubiquitous. This development raises a serious concern in developing countries like Nigeria because of poor personal and environmental hygiene aid the spread of *Salmonella* sp. through the faeco-oral route [28]. Multidrug resistant *Salmonella* sp. poses a threat to public health because it could result in therapy failure from the treatment of salmonellosis which has clinical symptoms that include fever, diarrhoea, vomiting and abdominal pain [29]. Antibiotic resistance of *Salmonella* sp. to ampicillin, ceftriaxone, cotrimoxazole, ciprofloxacin and chloramphenicol usually used for the treatment of enteric fever caused by *S. typhi* could pose a challenge to healthcare providers [30,31]. Previous studies have demonstrated that *Salmonella* spp. isolated from eggs, meat and egg products are resistant to many antibiotics. Recently, Rapid Alert System for Food and Feed (RASFF) raised a concern regarding the increasing spread of *Salmonella* sp. in foods other than meat. *Salmonella* sp. isolated from exposed yam flour not being sensitive to any of the antibiotics is a bigger challenge compared with the result reported by Mąka et al. [12] from a similar study in Poland. In Nigeria, drugs are commonly purchased without doctor’s prescription [5,32]. Abuse of antibiotics in developing countries like Nigeria compared with Poland could have contributed in the development of antimicrobial resistance of *Salmonella* sp. isolated from exposed plantain flour.

According to the International Commission on Microbiological Specification for Food (ICMSF), the safe limit of total viable count in food should not exceed 1 x 10⁵ cfu/g [6,33]. Standard Codex 176-1989 [34] as well as EAS 739:2010
recommend maximum total viable count and mould count of 5.00 log cfu/g and 3.00 log cfu/g, respectively as acceptable microbiological limits for cassava flour [4]. In terms of total viable count, exposed or packaged cassava, plantain and yam flour did not meet food requirement stipulated by ICMSF and Standard Codex. Total fungal count (TFC) of freshly processed yam flour reported by Omohimi et al. [6] is similar to the results obtained from exposed and packaged yam flour. However, TFC of yam flour from open markets and supermarkets reported by Omohimi et al. [6] were higher than results reported in this study. Agwa and Ossai-Chidi [22] reported that fungal count of cassava, yam and plantain flour processed industrially range between 2.9-3.2 x 10^3, 2.8-3.2 x 10^2 and 2.8-3.2 x 10^3 cfu/g whereas that of locally processed flour range between 3.4-4.2 x 10^3, 3.7-4.2 x 10^3, 3.1-4.9 x 10^3 cfu/g, respectively is in agreement with results obtained from this study. Increase in total viable count of stored cassava flour reported by Chukwu and Abdullahi [35] is in agreement with results from this study but the total fungal count of stored cassava flour is not. Their study revealed that total viable bacterial count increased from 1 to 29.0 x 10^4, 32.0 x 10^4, 31.0 x 10^4 cfu/g and 34.0 x 10^4 cfu/g while that of fungal count increased from about 1 to 400 x 10^3, 720 x 10^3, 310 x 10^3 cfu/g and 380 x 10^3 cfu/g in cassava flour control sample, sample A, sample B, and Sample C, respectively. The high microbial load of cassava, yam and plantain flour (exposed and packaged) reported in this study could be reduced to safe limits by subjecting the flours to heat treatments in the course of using the flours to produce diverse food products.

Shelf life study of the flour samples revealed that moisture content of packaged cassava flour and exposed yam flour stored for 2 Wks as well as packaged yam flour stored for 3 Wks was below 13% which is tolerable for stored dried foods. However, the moisture content of other stored flour samples (exposed and packaged) was above 13%. According to Omohimi et al. [6], moisture content of yam flour obtained from open markets and supermarkets range between 9.20-14.61%. This is in agreement with the results of this study. Ogundare-Akanmu et al. [36] reported that an increase in moisture content occurred during storage of plantain flour which ranged between 7.20-13.92%. Similarly, research findings by Lawal et al. [37] showed that packaged yam flour stored in polythene and Hessian bag experienced an increase in moisture content. Their study revealed that between Wk 0-6, yam flour stored in Hessian bag increased from 3.50-13.70% while that of polythene packaged yam flour increased from 3.50-14.80%. Uchechukwu-Agu et al. [38] reported that cassava flour from two cassava cultivars ‘TME 419’ and ‘UMUCASS 36’ experienced moisture reduction from 12.0-7.1% and 9.8-6.8%, respectively at different storage conditions.

5. CONCLUSION

Bacillus sp. and Staphylococcus sp. were isolated from exposed and packaged cassava, yam and plantain flour placed in open markets and supermarkets for public consumption. Escherichia coli and Salmonella sp were also present in exposed yam and plantain flour, respectively. Antibiotic susceptibility testing revealed that antibiotics generally effective against Gram-positive bacteria were more sensitive against the Gram-positive bacterial isolates when compared with the sensitivity of antibiotics generally effective against Gram-negative bacteria used against Gram-negative bacterial isolates. All the bacterial isolates were sensitive to at least one antibiotic except Salmonella sp. High microbial load and bacterial isolates from the flour samples being resistant to commonly used antibiotics calls for strict good manufacturing practices, minimal handling of the flours with bare hands and proper packaging of the product displayed in open markets and supermarkets.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


