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Bacteria of Public Health Significance on Fruit and Vegetables Sold Across Markets in Makurdi, Nigeria

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Abstract

Fruits and vegetables are composed of vitamins, minerals, and phytochemicals and are globally recommended as part of a healthy diet. But despite their immense nutritional benefits, their surfaces can become contaminated with pathogenic microbes leading to illness or death. We examined the surfaces of fresh garden egg (*Solanum aethiopicum*), carrot (*Daucus carota*), mangoes (*Mangifera indica*), and oranges (*Citrus sinensis*), purchased from the four major fruit markets in Makurdi (Railway, Wurukum, Wadata, and High-level) for bacteria of public health significance and also estimated the total aerobic plate count (TAPC). Samples were tested for the presence of bacteria by culturing on selected media. Characterization of the isolates was achieved using biochemical tests, Gram staining, and microscopy. Six different bacteria were detected -*Pseudomonas spp*., *Staphylococcus aureus*, *Klebsiella spp*., *Escherichia coli*, *Salmonella spp*., and *Streptococcus spp*., in the range 6.25×10^6 to 10.2×10^6 cfu/g in the sampled produce. Among the sampled markets, the fruits and vegetable purchased from High-level market revealed the highest contamination. Carrots purchased from High-level had the highest TAPC of 12.2×10^6 cfu/g, mangoes from Wadata market (9.0×10^6 cfu/g), while garden eggs and oranges also from High-level market showed 8.4×10^6 and 7.6×10^6 cfu/g respectively. At 36.6%, carrots had the highest percentage contamination, followed by mangoes (23.3%), while oranges and garden eggs were the least contaminated at 20% each. There are public health and hygiene risks regarding the fruits and vegetables sold in markets in Makurdi. Urgent steps to address these public health concerns are required.

Keywords

Food Microbiology, Bacterial Contamination, Fresh Produce, Food Safety, Fresh Fruits and Vegetables, Nigeria

1. Introduction

Fresh fruits and vegetables are components of a healthy and balanced diet [1, 2]. They provide an important source of carbohydrates, vitamins, minerals, electrolytes, fibers, and phytochemicals, particularly antioxidants [3]. Studies have found positive associations between a rise in the consumption of fruit and vegetable and a lower risk of developing chronic diseases [4], cancer and cardiovascular diseases [5, 6, 7, 8], and diabetes [9, 10]. Due to their health-promoting benefits and awareness, there has been an increase in consumption globally [11]. In Nigeria, there are no strict dietary recommendations or governing guidelines for the Required Daily Allowance (RDA) of fruits and vegetables. Nonetheless, the Ministries of Health, Agriculture, and Rural Development in collaboration with international agencies encourage the generous intake of any fruit and vegetables that are in season [12]. In many western countries, however, an increase in the daily intake of fruit and vegetable servings is promoted; 5 to 9 servings a day in America [13, 14] and 7+ portions daily in England [5].

Eating fruits and vegetables raw and uncooked maximize their health benefits, yet, if contaminated, may cause an increased risk of foodborne disease. For the reason that fresh fruits and vegetables have been reported to harbor microbial populations [1], some of which are vehicles for human pathogens. Globally, 600 million cases of foodborne illness and 420, 000 deaths have been reported [15]. The burden of disease is higher in under 5-year- olds resulting in 125 000 deaths yearly. Generally, viruses have been reported to be behind most foodborne illness; yet, bacteria pathogens, especially belonging to *Staphylococcus*, *Salmonella*, *Clostridium*, *Campylobacter*, *Listeria*, *Vibrio*, *Bacillus*, and *Escherichia coli* have been implicated in severe foodborne infections resulting in hospital admissions and mortalities [16, 17].

Several studies on the microbial safety and quality of fresh fruits and vegetables have been carried out in various parts of Nigeria. In a study in Kaduna State, *Staphylococcus aureus*, *Streptococcus spp*., *E. coli, Klebsiella spp*., *Enterobacter spp*., and *Citrobacter spp.,* were detected from selected fruits and vegetables [18]. In Oshogbo, Osun State, *Pseudomonas aeruginosa*, *S. aeureus*, *Enterococcus faecalis*, *Bacillius cereus*, *Listeria monocytogenes*, *Citrobacter spp.*, and *Candida spp.*, including *Entamoeba coli* cysts and ova of *Ascaris lumbricoides* were detected on various fruits and vegetables [19], while Eni et al. [20] in Sango-ota, Ogun State, identified *S. aureus, Staphylococcus spp., Klebsiella spp., Salmonella spp., Actinomycetes,* and *Escherichia coli*. Similarly, *Pseudomonas spp.,* and *Bacillus spp.,* was detected on lettuce in Edo metropolis [21]. In a study by Aguoru et al. [22] in Makurdi, oranges, garden eggs, avocado pears, carrots, and mangoes were contaminated with *S. aureus*, *E. coli*, *Klebsiella spp.*, *Pseudomonas aeruginosa*, *Streptococcus spp.*, *Salmonella spp.*, *Lactobacillus spp.*, and *Proteus spp.*, while studies by Obetta et al. [23], on stored apples, banana, carrots, and oranges in Makurdi, identified coliforms, mesophiles, molds and yeast as the contaminating microbes. The aim of this study is to identify and enumerate the bacteria associated with selected fresh fruits and vegetable sold in markets in Makurdi, to highlight any potential health hazards from the consumption of these produce.

2. Materials and Methods

2.1. Study Area

This study was carried out in Makurdi, Benue State, Nigeria.

2.2. Sample Collection

A total of forty (40) undamaged samples, comprising of ten samples each for oranges, garden eggs, carrots, and mangoes were purchased at random from traders at Railway (Figure 1), Wadata (Figure 2), High level, and Wurukum markets, all situated in Makurdi metropolis from April – June 2019. The fruits and vegetables tested in this study were selected based on two key factors (i) those that are eaten raw and (ii) their availability during the season of the year. On purchase, the samples were immediately packaged separately in plastic bags and transported to the Microbiology laboratory, Benue State University Makurdi within 24 hours and analyzed immediately.

Figure 1. *Display of oranges and carrots at Railway Market.*

Railway market is the major market for fruits and vegetables in Makurdi. As indicated, the seller is washing the carrots in a yellow plastic bowl, while the oranges are exposed directly to the sun and other contaminants.

Figure 2. *Wadata market showing garden eggs and salad vegetables on display.*

The salad vegetables on display include- cucumber, cabbage, green pepper, green beans, and carrots.

2.3. Bacteriological Examination of Fresh Produce

To suspend surface bacteria that might be present on the produce, 10 g of each whole sample (oranges, carrots, mangoes, and garden eggs) bought from each location was weighed and diluted in 90 ml of buffered peptone water (BPW) in a sterile tube and centrifuged at 10,000 rpm for 2 minutes before making dilutions. A 1 ml aliquot of the homogenate mixture was sampled to make 1:10 serial dilutions in BPW water and analyzed for microbes by culturing on nutrient agar and eosin methylene blue (EMB) agar using the spread plate. This was performed in duplicates. Inoculated plates were left on the workbench to solidify, after which they were incubated at 37°C for 24 hrs. For the enumeration of *E. coli* and other coliforms, the method by Feng et al. [24] was followed, and the culture was plated onto MacConkey agar plates. Pure colonies of bacteria were obtained by picking discrete colonies using a sterile wire loop and subculturing them onto freshly prepared agar plates. The plates were then incubated at 37°C for 24 hrs.

2.3.1. Gram Staining

Following the positive growth of culture on each agar plate, Gram staining was performed as a preliminary step in the initial classification of bacteria. The procedure by Smith and Hussey [25] was adopted.

2.3.2. Motility Test

To determine whether the isolated bacteria were motile or not, a microscopic analysis using the hanging drop method [26].

2.4. Biochemical Tests

Gram staining, microscopy, and biochemical tests namely catalase, oxidase, indole, and sugar fermentation were performed to enable bacterial identification [27, 28].

3. Results

Table 1 shows the results for the characterization and identification of bacteria from the test samples.

Table 1. *Results for Biochemical test and Characterization on the sampled fruits and vegetable.*

Biochemical Test				Gram reaction			
CA	TSI	Motility test	IND	+ve/-ve	Colony morphology	Microscopic morphology	Organism identified
+	-	-	-	+	Moist and yellow	Cocci in clusters	*Staphylococci spp.,*
-	-	-	-	+	Low convex discrete	Cocci in chains	*Streptococci spp.,*
+	+	+	+	-	Pinkish	Rod-shape	*E. coli*
-	+	-	-	-	Large and moist	Rod-shape	*Klebsiella spp.,*
+	-	+	-	-	Small, rough, oval and greenish yellow	Rod-shape	*Psuedomonas spp.,*
+	+	+	-	-	Black green colony	Rod-shape	*Salmonella spp.,*

Key:+ = positive; - = negative; CA signify catalase assay; TSI is Triple Sugar Iron assay; IND- Indole Test

From the biochemical tests, Gram reaction and microscopy, we identified – *Staphylococci spp.*, *Streptococci spp.*, *E. coli*, *Klebsiella spp.*, *Pseudomonas spp.*, and *Salmonella spp*.

Next, the total aerobic plate count (TAPC) from the sampled fruits and vegetable was estimated from the different markets. The results are displayed (Table 2).

Table 2. *Total Aerobic Plate Count for the test samples (cfu/g).*

Sample type	Sampled market	Total Aerobic Plate count	Average TAPC
Garden Egg	Wurukum	6.0×10^6	7.2×10^6
	Wadata	6.8×10^6	
	High-level	8.4×10^6	
	Railway	7.6×10^6	
Carrot	Wurukum	9.2×10^6	10.2×10^6
	Wadata	10.8×10^6	
	High-level	12.2×10^6	
	Railway	8.4×10^6	
Mango	Wurukum	6.4×10^6	7.4×10^6
	Wadata	9.0×10^6	
	High-level	6.8×10^6	
	Railway	7.4×10^6	
Orange	Wurukum	6.8×10^6	6.25×10^6
	Wadata	5.0×10^6	
	High-level	7.6×10^6	
	Railway	5.6×10^6	

All the markets recorded high bacteria load ($\geq 5\times10^6$) on all the samples. Carrots had the highest average TAPC of (10.2×10^6); followed by mangoes (7.4×10^6), garden eggs (7.2×10^6), while orange has the least count (6.25×10^6). The TAPC of the fruits and vegetable indicate that carrots purchased from High-level showed the highest TAPC (12.2×10^6 cfu/g), mangoes from Wadata market (9.0×10^6), garden eggs from High-level (8.4×10^6) and oranges from High-level market showed the least TAPC (7.6×10^6 cfu/g).

Evaluation of Percentage contamination of fresh produce

From the total number of produce purchased, the percentage contamination was analyzed. As presented (Figure 3), carrots, showed the highest contamination of 36.6%, whereas mangoes, oranges, and garden eggs were contaminated with 23.3%, 20% and 20% respectively.

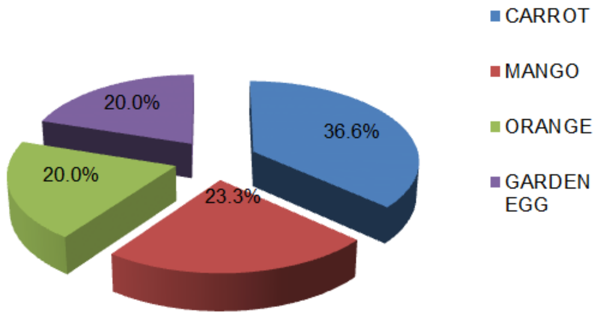

Figure 3. *Percentage contamination of purchased fresh produce.*

4. Discussion

Consumer demand for uncooked, organic fruits and vegetables sold in Makurdi has soared in recent years (M. Joseph, Personal communication, May 2019). Yet, data assuring their safety and microbial quality is limited. Data from Table 1 reveal that there are differences between the results obtained for biochemical tests, Gram reaction, colony morphology, and microscopic analysis. *S. aureus* was detected from all the produce in all the markets sampled (results not shown). This is unsurprising because the bacteria although found in the environment, also colonizes the skin of healthy humans as a normal flora [29]. The contact of bare skin with produce may have been a potential route for contamination. *Klebsiella spp.,* were also identified in the current study. The bacteria are widespread, and colonize multiple sites in the environment and the mucosal surfaces of mammals as the most prevalent habitats [30]. Our results also revealed *Streptococcus spp.*, and members of the genus may be pathogens, commensals, or cause opportunistic infections [31]. Infected food handlers may transmit *Streptococci* via food majorly [32], and this could have been the source of contamination of the sampled fresh produce. The presence of *E. coli* in the present study is worrisome, as it is an indicator of fecal contamination. *Pseudomonas spp.*, and *Salmonella spp.*, was present in the produce sampled.

As indicated, (Table 2), the TAPC from the produce tested namely garden eggs, carrots, mangoes, and oranges were high, and this was recorded for all the sampled markets, especially at High-level market. This suggests an unsatisfactory bacteriological quality that could pose a health challenge when consumed without further decontamination. The high bacterial contamination obtained from our study is in agreement with studies conducted [32]. In their study, for some edible fruits sold in Makurdi, high bacteria count was detected ranging from 6.4×10^7 to 9.0×10^7 cfu/g. However, there are some differences in the produce and markets sampled. Similarly, studies performed by [20] in Sango Ota, Nigeria also found high microbial contamination ranging from 3×10^6 - 1.82×10^7 cfu/ml. Studies by Akoachere and colleagues on salad vegetables including carrots in Cameroon disclosed high bacteria count of 2.5×10^6 to 15×10^6 cfu/g [33]. These results suggest that problems of food safety cut across the African continent.

It has been recommended that fruits and vegetables intended for sale should be thoroughly washed in potable chlorinated water to remove soil and debris. This enables the destruction and/or decline in the levels of microbes of public health significance [34, 35, 36]. Hence, the use of chlorine could be replaced by substitutes like chlorine dioxide (ClO_2), Acidified sodium chlorite (ASC $NaClO_2$), ozone, electrolyzed water [37] can also be utilized. Water guard, composed of sodium hypochlorite and used to treat water before drinking is locally available and could be used to decontaminate fresh produce before sale. In Makurdi, fruits and vegetables sold in the markets are generally not subjected to extra processing steps to ensure the removal or inactivation of pathogenic microorganisms or chemicals before they are sold to the consumers (M. Joseph, Personal communication, June 2019). When the produce is washed, the water used is not filtered and treated to guarantee that it meets the chemical and microbiological requirements for food safety, as practiced globally.

5. Conclusion

The results obtained from this study reveal that all the sampled fruits and vegetables sold in markets in Makurdi were contaminated with pathogenic bacteria (*Pseudomonas spp.*, *Staphylococcus spp.*, *Klebsiella spp.*, *E. coli*, *Streptococcus spp.*, and *Salmonella spp.*,) and the bacterial load was beyond the permissible limits. Contamination could be a result of poor sanitary conditions in and around the markets, and poor agronomic practices during cultivation, harvesting, transportation, and storage. This is a massive public health challenge that calls for the implementation of food safety laws to safeguard public and individual health. Furthermore, Government agencies such as the Ministries of Environment and Health should ensure awareness by organizing workshops to educate retailers on good agricultural practices during cultivation to improve the microbial quality of fresh produce.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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