Original Research Article

Assessment of Spoilage and Pathogenic Bacteria in Fruits and Vegetables from Retail Sources and Home-gardens

Abstract: Background and Objective: Consumption of fruits and vegetables which are known to be highly nutritious have been linked to disease outbreaks which constitute food safety and public concern. This study aimed to assess the safety of selected fruits and vegetables from fruit markets and home gardens, within the South-West region of Nigeria. **Methods**: Fifty-three (53) samples of watermelon, cucumber, tomatoes and garden-eggs were collected and subjected to microbiological analysis. Isolated bacteria were screened for their pathogenicity and spoilage potential. **Results**: A total of 146 bacteria were isolated, 75 (45.7 %) were from retail samples and 71 (43.3 %) from home-garden. The genera: *Bacillus* (15.9 %), *Corynebacterium* (11.0 %), *Lactobacillus* (1.2 %), *Listeria* (1.8 %), *Staphylococcus* (12.8 %), *Enterococcus* (1.2 %), *Micrococcus* (1.2 %), *Acinetobacter* (3.7 %), *Aeromonas* (2.4 %), *Alcaligenes* (0.6 %), *Brucella* (0.6 %), *Vibrio* (0.6 %), and the family *Enterobacteriaceae* (36.0 %) were identified. Isolates with haemolytic potentials were 51 (31 %) while 49 (30 %) had the ability to cause spoilage. **Conclusions**: The overall microbiological quality and safety of fruits and vegetable samples analysed in this study is low, as they were contaminated by diverse pathogenic, and spoilage microorganisms. The presence of these pathogens in retailed and home garden fruits and vegetables is a pointer to public health risks and food safety threats. Hence, the need for improved hygienic practice.

Keywords: fruits; vegetables; pathogens; public health; food safety

1. Introduction

Fruits and vegetables pose numerous benefits to humans, their daily consumption have been advocated as a necessity for maintaining good health as they constitute an important source of dietary fibre, vitamins and minerals [1]. They have a distinctive nutrient profile that is essential in maintaining body fitness, skin rejuvenation and diseases prevention. Fruits and vegetables are life enhancing medicines packed with vitamins (such as vitamin C, vitamin B-complex and vitamin A), minerals (such as calcium, sodium, potassium, and magnesium), phytochemicals (such as phenolics, flavonoids and carotenoids), antioxidants, dietary fibres and other nutrient compositions that are essential for human health [2]. They also contribute to lowering risk of diseases such as cardiovascular diseases and even onset of cancer [2]. Apart from the nutritional benefits, today's never-ending list of activities has forced individuals to be busy all of the time, altering their normal diets and prompting a shift to fresh fruits and vegetables to save more time [3].

The indigenous microbial composition of fresh fruits and vegetables is generally non-pathogenic [4]. The innermost tissues of healthy fruits and vegetables are devoid of microorganisms, however, the external surfaces of raw fruits and vegetables can be contaminated with diverse microorganisms, depending on the microorganisms present in the environment where they were harvested, the condition of the fruits and vegetables, handling methods, and storage time and conditions [5]. As a result, these fruits and vegetables can cause diseases even while still on the plant in fields or orchids, as well as during harvesting, transportation, distribution, and marketing, and at even home [5].

Besides, the water activity of fruits and vegetables which is known to be high, and pH of nearly neutral (in vegetables) and low acidic (in many fruits) tissues, promotes swift microbial development, these characteristics create an ideal environment for a variety of human pathogens to contaminate fruits and vegetables [4].

Foodborne infection and poisoning linked with fresh fruits and vegetables is emerging due to increased consumption and increased consumer awareness of their nutritional values and convenience. Fruits and vegetables, particularly the ones eaten raw, have been a source of foodborne disease outbreaks such as typhoid fever, dysentery, diarrhea and even cholera [6]. In 2022, the World Health Organization (WHO) recorded 600 million foodborne infection cases worldwide, with 420,000 fatalities related to foodborne infection. Most of these foodborne infections are caused by foodborne pathogens, some of which were from fresh fruits and vegetables [7].

Foodborne outbreaks from pathogens associated with fruits and vegetables which are known to be highly nutritious are of food safety concern. Hence, this research was aimed at detecting bacterial pathogens associated with fruits and vegetables; on farms (home-gardens) and from fruit markets (retail) within the South-western region of Nigeria.

2. Materials and Methods

2.1. Sample Collection

Fruits and vegetables that grow close to the soil and are mostly eaten raw were selected for this study. Selected fruit samples (cucumber, watermelon, garden egg and tomato) were collected from different markets and home gardens from Oyo State, Ogun State and Ekiti State of the South-western region of Nigeria. A total number of 53 samples were collected, 3 watermelons, 5 cucumbers, 29 tomatoes and 16 garden eggs samples. The samples were collected aseptically into sterile bags and labelled accordingly before being taken to the Microbiology Laboratory, Ajayi Crowther University for Microbiological analyses.

2.2. Enumeration of Bacteria

The mesocarp and epicarp of each sample was randomly cut out, weighted (1 g), transferred into a sterile stomacher bag (Seward, UK) and homogenized in 9 mL sterile water for 2 minutes using a stomacher (Stomacher 80 Biomaster, Seward, UK). Serial dilution was carried out on the resulting homogenate using sterile water and was pour plated on Nutrient Agar (NA) (HiMedia, India), Brain heart infusion agar (BHIA) (HiMedia, India), MacConkey Agar (MCA) (HiMedia, India), Eosin Methylene Blue Agar (EMBA) (HiMedia, India), and Salmonella-Shigella Agar (SSA) (HiMedia, India) in duplicates. The plates were incubated aerobically at 37 ºC for 24 h. The bacterial count was evaluated based on media used, such as aerobic mesophilic count (NA), *Listeria* (BHIA), Enterobacteriaceae (MCA), *E. coli* (EMBA) and the presence of *Salmonella* and *Shigella* (SSA). After incubation, the viable organisms were enumerated and average colony forming units per gram (CFU/g) were calculated.

2.3. Identification of Isolates

All bacterial isolates obtained from this study were identified using morphological and biochemical characteristics. Gram staining, endospore staining, oxidase, catalase, citrate, indole, methyl red, Voges Proskauer, carbohydrate fermentation, growth in 6.5 % NaCl and 0.75 % KCN were performed following the standard protocols. Motility test, H_2S and gas production was carried out by stabbing triple sugar iron (HiMedia, India) agar vertically with the bacterial isolates and incubated for 24 – 48 hours at 37 ºC [8, 9].

2.4. Determination of Haemolysin and Amylase Production in Isolates

Haemolysis and amylase assays were carried out on all isolates to obtain haemolysin and starch hydrolyzing organisms potentials respectively. Haemolysis assay was achieved on freshly prepared blood agar (10 % human blood free of antibiotics in 100 mL nutrient agar; v/v) and the amylase test was carried out using starch agar (w/v: 0.5 % peptone, 0.3 % yeast extract, 1.5 % agar-agar, 0.2 % soluble starch) [10].

3. Results

3.1. Total Viable Count of Samples

The total viable count of bacteria in selected retail fruits and vegetables as well as those from home-gardens were evaluated. Total aerobic mesophilic bacterial count (CFU/g) ranged from 3.0×10^2 from retail watermelon to 1.90×10^4 from home-garden tomato. Bacterial count (CFU/g) from retail samples ranged from 3.0×10^2 from watermelon to 8.84×10^3 from garden-egg while that of home-garden samples ranged from 1.78×10^3 from watermelon to 1.90×10^4 tomato (Table 1).

3.2. Occurrence of Bacterial Isolates and their Characteristics

A total of one hundred and forty-six (146) bacteria were isolated from retail and home-garden fruits and vegetables, 75 (45.7 %) were from retail samples and 71 (43.3 %) from home garden samples. From the 146 total bacteria isolated, 74 (50.7%) were Gram positive in which 49 (33.6%) were rods and 25 (17.1%) were cocci, 72 (49.3%) were Gram negative, all rods. The bacteria isolated were grouped based on their Gram reaction and morphology; Gram positive rods, Gram positive cocci, and Gram-negative rods. The biochemical tests carried out on isolates with respect to their Gram reaction and morphology and their probable identity are presented in Table 2a, 2b and 2c.

3.3. Distribution and Frequency of Occurrence of Bacterial Isolates

The frequency of occurrence for the different bacteria isolated in both retail and home garden samples are presented in Table 3. *Enterobacter intermedius* had the highest number of occurrence (11.0%) and it occurred more in retail samples than in home garden samples, followed by *Staphylococcus aureus* (10.2%) in which more also occurred in retail samples than in home garden samples. Frequency of occurrence of *Bacillus cereus* was 8.9% in which more occurred in retail samples than in home garden samples. Bacterial species with the least frequency of occurrence include; *Bacillus pantothenticus, Bacillus licheniformis, Micrococcus luteus, Micrococcus varians, Enterobacter cloacae, Erwinia chrysanthemi, Salmonella bongori, Salmonella enterica. Serratia rubidaea, Serratia fonticola, Vibrio cholerae* and *Yersinia pestis* (0.7%) in which the majority occurred more in retail samples than in home garden samples

3.4. Production of Haemolysin and Amylase in Bacterial Isolates

The haemolysis assay carried out on all isolates resulted in classification of the 146 bacterial isolates into either α-haemolytic, β-haemolytic or γ-haemolytic. A total number of 101 (69.2%) (46 from retail and 55 from home garden samples) bacterial isolates were γ-haemolytic, only 2 (1.4%) (1 from retail samples and 1 from home garden samples) were α-haemolytic, while 43 were β-haemolytic (29.4%) (28 from retail samples and 15 from home garden samples) (Figure 1).

For the amylase assay, only 38 (27.1%) (24 from retail samples and 14 from home garden samples) of the 146 screened bacterial isolates were positive for amylase production as shown in Figure 1.

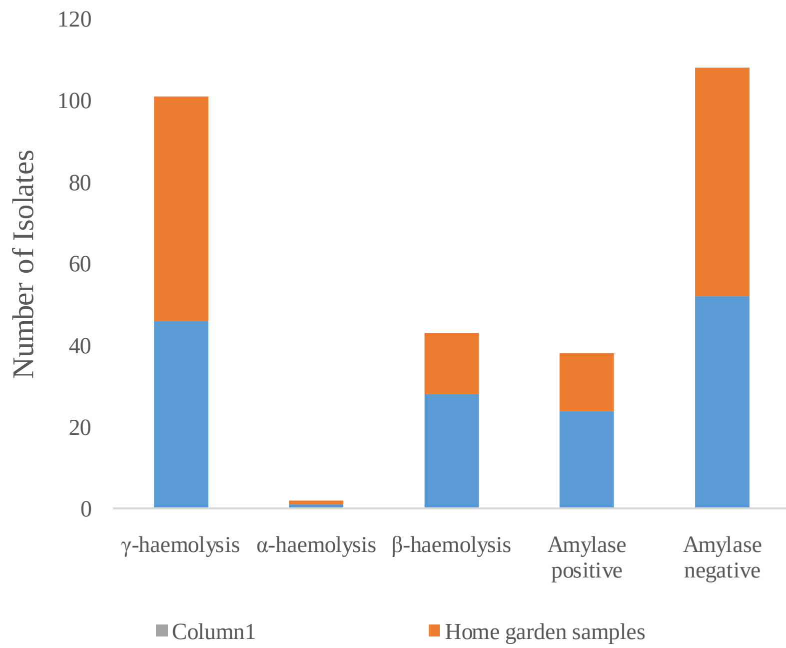

Figure 1. Production of haemolysin and amylase in bacterial amylase from retail and home-garden fruits and vegetables

Table 1. Colony counts of home-garden and retail fruits and vegetables samples

Colony Forming Units/ g	Cucumber		Watermelon		Garden-egg		Tomato	
	Retail	Home-garden	Retail	Home-garden	Retail	Home-garden	Retail	Home-garden
Aerobic mesophilic count	1.46×10^3	4.5×10^3	3.0×10^2	1.78×10^3	8.84×10^3	4.8×10^3	3.4×10^2	1.90×10^4
Listeria count	2.15×10^3	8.44×10^3	8.45×10^2	6.50×10^2	2.92×10^4	3.97×10^3	1.35×10^3	9.44×10^3
Salmonella and *Shigella* count	3.0×10^1	-	2.0×10^1	-	6.47×10^3	3.90×10^3	2.8×10^2	1.28×10^4
Enterobacteriaceae count	3.0×10^1	1.44×10^3	2.0×10^1	-	1.27×10^4	1.04×10^4	2.95×10^2	2.60×10^4
E. coli count	2.0×10^1	1.22×10^3	-	-	6.6×10^4	3.05×10^3	3.8×10^2	1.74×10^4

Table 2a. Characteristics showing the identity of Gram-positive rod-shaped bacterial isolates from retail and home garden fruits and vegetables

Characters	*Bacillus azotoformans*	*B. brevis*	*B. cereus*	*B. licheniformis*	*B. pantothenticus*	*Bacillus* spp.	*C. kutscheri*	*C. xerosis*	*Lactobacillus fermentii*	*Listeria monocytogenes*
Number of Isolates	2	2	13	1	1	7	9	9	2	3
Endospore	+	+	+	+	+	+	-	-	-	-
Catalase	-	+	+	+	+	+	+	+	-	+
Motility	+	+	+	+	+	+	-	-	-	+
Gas production	-	-	-	-	-	-	-	-	-	-
H_2S production	-	-	-	-	-	-	-	-	-	-
Citrate	+	-	+	+	+	+	-	-	-	+
Growth in 6.5 % NaCl	-	+	+	+	-	+	+	+	+	+
Methyl red	-	-	-	-	-	+	-	-	-	+
Voges Prokauer	-	-	+	+	-	+	-	-	-	+
Urease	NA	NA	NA	NA	NA	NA	NA	NA	NA	-
Starch	-	+	+	+	+	-	+	-	+	-

Haemolysis	-	-	+	-	+	+	+	+	-	+
Carbohydrate utilization										
Glucose	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	-	+	-	-	-	+	+	+
Sucrose	+	+	+	+	-	-	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	-
Mannitol	+	-	-	+	-	-	-	+	-	-
Arabinose	+	-	-	+	-	+	-	+	+	-
Xylose	+	-	-	+	-	-	-	-	+	-

KEY: NA- Not applicable

Table 2b. Characteristics showing the identity of Gram-positive cocci-shaped bacterial isolates from retail and home garden fruits and vegetables

Characters	*Enterococcus* spp.	*Micrococcus luteus*	*Micrococcus varians*	*Staphylococcus aureus*	*Stapylococcus* spp.
Number of Isolates	2	1	1	15	7
Catalase	-	+	+	+	+
Yellow Pigmentation	-	+	+	-	-
Starch	-	-	-	+	-
Haemolysis	-	-	-	+	-
Carbohydrate Fermentation					
Glucose	+	-	+	+	-
Mannitol	+	-	-	+	-

Table 2c. Characteristics showing the identity of Gram-negative rod-shaped bacterial isolates from retail and home garden fruits and vegetables

Bacteria	Number of Isolates	Oxidase	Indole	Growth in 0.75%	Methyl	Voges Proskauer	Citrate	Gas	H_2S	Motility	Starch	Haemolysi	Carbohydrate Fermentation			
													Glucose	Lactose	Sucrose	Maltose
Acinobacter baumanii	6	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-

Aeromonas hydrophila	1	+	+	NA	+	+	+	+	-	+	+	+	+	-	+	NA
Aeromonas spp.	3	+	+	+	-	-	+	-	-	-	+	-	+	-	-	-
Alcaligenes faecalis	1	+	-	NA	-	-	+	-	-	+	-	+	-	+	-	-
Brucella melitensis	1	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-
Citrobacter diversus	5	-	+	NA	-	-	+	-	-	-	-	-	+	+	+	NA
Enterobacter aerogenes	2	-	-	NA	-	+	+	+	-	+	-	-	+	+	+	NA
Enterobacter cloacae	1	-	-	NA	-	+	+	+	-	+	+	-	+	+	+	NA
Enterobacter intermedius	16	-	-	NA	+	+	+	+	-	+	+	-	+	+	+	NA
Erwinia cacticida	2	-	-	NA	-	+	+	-	-	+	+	+	-	-	-	NA
Erwinia chrysanthemi	1	-	+	NA	-	+	+	+	+	+	-	-	+	+	+	NA
Escherichia coli	3	-	+	NA	+	-	-	+	-	+	-	-	+	+	+	+
Klebsiella oxytoca	9	-	+	+	-	+	+	+	-	-	-	-	+	+	+	+
Klebsiella pneumonia subsp. *Ozaenae*	8	-	-	+	-	+	+	+	-	-	-	-	+	+	+	+
Salmonella bongori	1	-	-	+	+	-	+	-	+	-	-	-	+	-	-	+
Salmonella enterica	1	-	+	-	+	-	+	-	+	-	+	+	+	-	-	+
Serratia fonticola	1	-	-	NA	+	-	+	-	-	-	-	+	+	+	+	NA
Serratia marcescens	4	-	-	+	-	+	+	-	-	+	+	-	+	-	+	+
Serratia rubidea	1	-	-	NA	-	+	+	-	-	-	+	-	+	+	+	NA
Shigella flexneri	3	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-
Vibrio cholera	1	+	+	NA	-	+	+	-	-	+	+	+	+	+	+	+
Yersinia pestis	1	-	-	-	+	-	-	-	-	-	-	-	+	-	-	+

4. Discussion

Fruits and vegetables have been and would continue to be widely consumed not only in Nigeria but across the globe and there will be need for continuous monitoring of the microbial safety of these agricultural produce based on diverse cultivation methods and handling techniques. The safety study investigated in this work showed that there was high aerobic mesophilic count ranging from 3.0×10^2 to 1.90×10^4 among the fruits and vegetable investigated, this is slightly lower than the report of Dobo [11]. The specific bacterial count is considerably reduced to the report of Rida and Deeba [12].

The occurrence of high numbers of viable bacteria, especially pathogens arose safety consciousness. The microbial load could be a function of poor storage conditions, unhygienic handling and prolonged time of storage of the fruits and vegetables, and this can increase the possibility of produce spoilage, as well as that of agricultural fruits and vegetables-associated outbreaks [13]. Aside watermelon, the total viable count of bacteria isolated from home garden samples were higher than that of retail samples. The increased total viable count of home garden samples might be due to the freshness of the samples and less damage to external surface. And the low viable count in retail samples could be as a result of several contacts during long storage and marketing accounting for the reduction of viable counts in retail fruits and vegetables [14]. Also, some environmental conditions and response mechanisms to stress might contribute to a decrease in the total viable count of microorganisms in the retail samples [15]. From the fruits and vegetables analysed in this study, tomatoes had the highest microbial load, followed by garden-egg, cucumber then watermelon.

It was noted that both home garden and retail samples were contaminated by various pathogens. However, the retail samples were comparatively contaminated with more diverse pathogens of different genera than the home garden samples. This report correlated with Ajayi *et al.* [16] and Dada and Olusola-Makinde [17] who gave report on contamination of vegetables by microorganisms from retailers in South-Western Nigeria. All microorganisms isolated from this study have previously been isolated from fruits and vegetables in other studies and researches both within Nigeria and other parts of Africa [10, 18, 19].

This study showed the presence of pathogenic bacteria with *Bacillus* species being the most dominant bacteria. *Bacillus* species form spores that are resistant to several unfavourable conditions such as cold, heat and even common disinfectants which is according to Gu *et al.* [20]. Some *Bacillus* species such as *Bacillus cereus* which was detected in this study have been implicated in food borne illness. It is mostly present in soil and may cause contamination of fruits and vegetables during harvesting. Their presence and their toxins could lead to diarrhoea (enterotoxins) or vomiting (Emetic/cereulide toxin) [21]. Contamination of fruits and vegetables with *B. cereus* is an emerging health safety concern as they can cause intoxications shortly after consumption, thus presenting serious health risk to consumers.

The detection of *Listeria monocytogenes* in this study notably in cucumber, watermelon from retail source and tomatoes from home-garden give concerns for health challenges. Several outbreaks of listeriosis have been reported originating from consumption of fresh produce [25] and its prevention is now a food safety challenge. *Staphylococcus aureus* was also dominant in both retail and home garden samples. This is an indication of poor hygienic practices by both farmers and handlers. *S. aureus* is a pathogenic organism and of public health concern which is according to the report of Ajayi *et al.* [8].

Among the *Enterobacteriaceae* isolated in this study *Enterobacter* and *Klebsiella* were found to be predominant. Other member of the family isolated in this study include *Shigella, Escherichia, Citribacter,* and *Serraia.* This result correlates with that of Oyedele *et al*. [10] where similar bacteria were isolated from fruits. The occurrence of these bacteria in fruits and vegetables collected from retail source could be as a result of contamination from the soil especially in cases where manure was used for the cultivation. The other routes of contamination can be from the method of irrigation and handling. Domestic waste water are often used for irrigation in home-gardens, this water might have been contaminated and could have accounted for the contamination with *Enterobacteriaceae* of fruits and vegetables collected from home-gardens. Clinically, several members of the family, *Enterobacteriaceae* are listed among the causative agent of gastrointestinal related cases [22] from the consumption of fresh fruits and vegetables. These findings highlight the critical importance of adequate monitoring of fruits and vegetable food chain to reduce contamination by and overgrowth of microorganisms, and ultimately, protect public health.

Microbial cells that synthesize the enzyme haemolysin have the ability to degrade or lyse red blood cells, indicating that they have pathogenic potential [23]. The occurrence of haemolytic bacteria in this study emphasizes the potential health concerns connected with the intake of ready-to-eat fruits and vegetables. Amylase enzyme activity is crucial for food deterioration, especially in sugar-rich foods like fruits and vegetables [24]. Approximately 27% (53) of the bacterial isolates examined in this investigation produced amylase, indicating their propensity to cause fruit deterioration. These haemolytic amylase-producing species provide an underappreciated risk to fruit and vegetable consumers because they can stick to and multiply in the gastrointestinal tract, resulting in severe gastroenteritis [10].

5. Conclusions

This study revealed that the fruits and vegetables samples from retail source and home gardens, analysed in this study were contaminated by diverse pathogenic and spoilage bacteria. The overall microbiological quality and safety of the fruits and vegetables from both retail source and home gardens is low, this implies that consumption of these produce may pose a threat to the general public, since they are essential part of daily diet, and this is a pointer to public health risks and food safety threats.

Good hygiene practice and improved agricultural processing with good sanitation are extremely recommended for individuals involved in fruits and vegetables handling in order to reduce fruits and vegetables contamination by microorganisms in general. Measures should be taken in sensitizing fruits and vegetable handlers and the general public on the hazard and risks associated with high microbial contaminated fruits and vegetables if these measures are not considered.

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