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

Abstract

Consumption of fruits and vegetables which are known to be highly nutritious has been linked to foodborne 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. A total of 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 using haemolysis and amylase production tests respectively. A total of 146 bacteria were isolated, 75 (45.7 %) were from retail samples and 71 (43.3 %) from the 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 %) could cause spoilage. The overall microbiological quality and safety of fruit 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 through training handlers along the supply chain.

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

1. Introduction

Fruits and vegetables pose numerous benefits to humans, and their daily consumption has 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 disease 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 the risk of diseases such as cardiovascular diseases and even the onset of cancer [2]. Apart from the nutritional benefits, today's never-ending list of activities has forced individuals to be busy all the time, altering their normal diets and prompting a shift to fresh fruits and vegetables to save more time [3]. Therefore, this practice eventually exposes the consumers to the health implications that could be associated with fruits and vegetables.

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 from, the condition of the fruits and vegetables, handling methods, and storage time and conditions [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]. Bacterial contamination of fruits and vegetables has been reported by several researchers within and outside Nigeria [5, 10, 11, 12, 16, 17, 21].

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 with 420,000 deaths globally. 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. While several research has been carried out on the contamination of fruits and vegetables, there is no report on the contamination of those from home gardens. 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 Southwestern 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 Southwestern region of Nigeria. A total number of 53 samples were randomly collected and subjected to the size of the fruits and vegetables with 3 watermelons, 5 cucumbers, 29 tomatoes and 16 garden eggs samples. The samples were collected aseptically into sterile bags and labelled with sample name, date and location 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 were 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. 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 were carried out on isolates with respect to their Gram reaction and morphology and their probable identity was determined. 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 were 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' potential 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 aerobic mesophilic bacterial count (CFU/g) of retail samples was higher than that of the home garden in most of the fruits and vegetables analysed. Cucumbers from retail source had a total viable bacterial count of 1.46×10^3 while the ones from the home garden have a count of 4.5×10^3. Watermelon and garden egg also followed the same pattern. Only tomatoes had a higher count of 8.84×10^3 CFU/g from the retail source samples than the count (4.8×10^3) from home-garden samples (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 %) were 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 (Table 2a, b and c).

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 is 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%) which also occurred more in retail samples than in home garden samples. The frequency of occurrence of *Bacillus cereus* was 8.9% which occurred more 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.

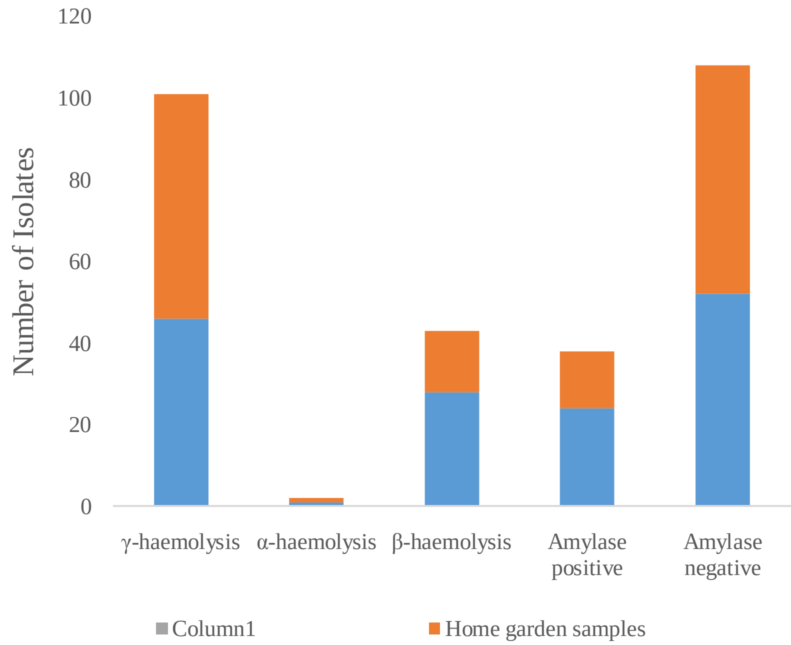

Figure 1. Production of haemolysin and amylase in bacterial amylase from retail and home-garden fruits and vegetables

Table 1. Colony counts (cfu/g) of selected home-garden and retail fruits and vegetables samples

Bacteria species	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	6
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	-	-	-	+	-	-	-	-	-	-	-	+	-	-	+

Table 3. Distribution and frequency of occurrence of bacterial isolates from retail and home-garden fruits and vegetables

S/N	Bacteria Isolates	Number of Occurrence			Percentage (%)
		Commercial Samples	Home Garden Samples	Total	
1	*Bacillus brevis*	1	1	2	1.4
2	*Bacillus cereus*	7	6	13	8.9
3	*Bacillus azotoformans*	1	1	2	1.4
4	*Bacillus pantothenticus*	1	0	1	0.7
5	*Bacillus licheniformis*	1	0	1	0.7
6	*Bacillus* sp.	7	0	7	4.8
7	*Corynebacterium kutscheri*	5	4	9	6.1
8	*Corynebacterium xerosis*	5	4	9	6.1
9	*Lactobacillus fermentii*	0	2	2	1.4
10	*Listeria monocytogenes*	2	1	3	2.0
11	*Staphylococcus aureus*	6	9	15	10.3
12	*Staphylococcus* sp.	1	5	6	4.1
13	*Enterococcus* sp.	1	1	2	1.4
14	*Micrococcus luteus*	1	0	1	0.7
15	*Micrococcus varians*	1	0	1	0.7
16	*Aeromonas hydrophila*	1	0	1	0.7
17	*Aeromonas* sp.	2	1	3	2.0
18	*Acinetobacter baumannii*	3	3	6	4.1
19	*Alcaligenes faecalis*	1	0	1	0.7
20	*Brucella melitensis*	0	1	1	0.7
21	*Citrobacter diversus*	1	4	5	3.4
22	*Escherichia coli*	1	2	3	2.0
23	*Enterobacter cloacae*	1	1	1	0.7
24	*Enterobacter intermedius*	12	4	16	11.0
25	*Enterobacter aerogenes*	2	0	2	1.4
26	*Erwinia chrysanthemi*	0	1	1	0.7
27	*Erwinia cacticida*	1	1	2	1.4
28	*Klebsiella pneumonia* subsp. *Ozaenae*	2	6	8	5.5
29	*Klebsiella oxytoca*	2	7	9	6.1
30	*Salmonella bongori*	1	0	1	0.7
31	*Salmonella enterica*	1	0	1	0.7
32	*Serratia marcescens*	1	3	4	2.7
33	*Serratia rubidaea*	0	1	1	0.7
34	*Serratia fonticola*	0	1	1	0.7
35	*Shigella flexneri*	3	0	3	2.0
36	*Vibrio cholera*	1	0	1	0.7
37	*Yersinia pestis*	0	1	1	0.7

4. Discussion

Fruits and vegetables have been and will continue to be widely consumed not only in Nigeria but across the globe and there will be a need for continuous monitoring of the microbial safety of this agricultural produce based on diverse cultivation methods and handling techniques. The safety study investigated in this work showed high aerobic mesophilic count ranging from 3.0×10^2 to 1.90×10^4 among the fruits and vegetables investigated (Table 1), 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 from watermelon, the total viable count of bacteria isolated from home garden samples was 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 the 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 reported on the contamination of vegetables by microorganisms from retailers in South-Western Nigeria. They isolated *Staphylococcus* spp., *Enterobacter* spp., *Salmonella* spp., *Shigella* spp. among other bacteria. All microorganisms isolated from this study have previously been isolated from fruits and vegetables in other studies and research 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 a 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. This organism is a foodborne pathogenic bacterium that causes a rare but dangerous infection called listeriosis, which its severity can cause infections ranging from mild gastroenteritis to severe blood and/or central nervous system infections, as well as abortion in pregnant women [25]. Several outbreaks of listeriosis have been reported originating from the 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 members 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 the retail source could be a result of contamination from the soil, especially in cases where manure was used for cultivation. The other routes of contamination can be from the method of irrigation and handling. Domestic wastewater is 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 the fruits and vegetable food chain to reduce contamination by an overgrowth of microorganisms, and ultimately, protect public health.

Microbial cells that synthesize the enzyme haemolysin can degrade or lyse red blood cells, indicating that they have the 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 (Figure 1), 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 an essential part of the daily diet, and this is a pointer to public health risks and food safety threats.

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

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