Assessment of bacteriological quality of fruits and vegetables sold in Gombe metropolitan market

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Abstract	Article History
Fruits and vegetables are important components of human diet that could be contaminated by different kinds of microorganisms. Microbial contamination may occur during any steps of transportation or processing from farm-to-table due to many environmental, animal, and human sources as well as technological applications. This study aimed at assessing the bacteriological contaminants of fruits and vegetables sold in Gombe metropolitan. A total of 30 fruits and vegetables samples were purchased from different vendors and transported to the Microbiology Laboratory for processing. Ten-fold serial dilution was performed on each sample and aliquots of 10^{-1}-10^{-3} dilutions were inoculated on Nutrient Agar and MacConkey Agar plates using pour-plate technique. The plates were incubated for 24 hours at 37°C and then observed for aerobic mesophilic and faecal coliform counts. The bacterial loads of the samples ranged from 3.7×10^4 to 2.7×10^5 and 2.9×10^4 to 8.7×10^4 for total mesophilic and faecal coliform counts, respectively. Subsequently, a total of eight bacteria were isolated and identified as *Escherichia coli, Salmonella* spp*., Klebsiella* spp*., Enterobacter* spp*., Pseudomonas* spp*., Staphylococcus aureus, Bacillus cereus,* and *Shigella* spp. In conclusion, the fruits and vegetables were found to be contaminated with different bacterial species of public health concern.	

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1.0 Introduction

There is a strong relationship between the consumption of fruits and vegetables and health of an individual (Abadias *et al.,* 2008). Raw fruits and vegetables may serve as sources of proteins, carbohydrates, vitamins and minerals which are essential in healthy human life. Vegetables are an important source of dietary fiber, vitamins and minerals, and have low energy density and provide a range of nutrients that are required to regulate the body metabolism. For these reasons, dietary guidelines recommend a high intake of vegetables (WHO/FAO, 2008). Various types of fruits, raw eaten vegetables and salads are very popular due to their attributes. In many countries, the consumption rates of raw fruits and vegetables are increasing every day. To prevent cancer, diabetes, heart disease, obesity and micronutrient deficiencies, joint FAO/WHO Expert consultation Panel recommends an adequate daily intake of 400–500g of fruit and vegetables (Soon *et al.,* 2012).

The importance of fruits and vegetables in nutritious and healthy diets is well recognized, and consumers have been encouraged to eat more of these products (WHO, 2012). The consumption of vegetables is

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highly relevant for a balanced diet as it leads to a healthier life style. In fact, the intake does not only reduce the development of disease, but also their fibers regulate the digestive functions of the human body (de Moura *et al.,* 2014).

Fresh produce may be contaminated with different kinds of microorganisms (Berger *et al.,* 2010). For instance, fruits and vegetables are widely exposed to microbial contamination through soil, dust, water and by handling at harvest or during postharvest processing (Carmo *et al.,* 2004; Verhoeff-Bakkenes *et al.,* 2011). Differences in microbial profiles of various fruits and vegetables result largely from unrelated factors such as resident microflora in the soil, application of nonresident microflora via animal manures, sewage or irrigation water, transportation and handling by retailers (Ray and Bhunia, 2007; Ofor *et al.,* 2009). In developing countries such as Nigeria, continued use of untreated waste water and manure as fertilizers for the production of fruits and vegetables is a major contributing factor to contaminations (Amoah *et al.,* 2009).

Fresh produce could serve as a vehicle for transmission of enterotoxigenic and enterohemorrhagic Escherichia coli, Salmonella species, Listeria monocytogenes, and thermotolerant Campylobacter specie, parasitic and viral pathogens capable of causing human illness (Abadias *et al.,* 2008; Aycicek *et al.,* 2006; Verhoeff-Bakkenes *et al.,* 2011). A number of reports showed that raw fruits and vegetables harbour potential food borne pathogens (Beuchat, 2002; Warriner *et al.,* 2009). Pathogenic E. coli and Salmonella species are involved in large food borne outbreaks worldwide, causing symptoms of gastroenteritis and chronic infections. Listeria monocytogenes is a psychrotolerant and ubiquitous microorganism that causes listeriosis and contaminate ready to eat vegetables (De Oliveira *et al.,* 2011). Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus species, Proteus species, Lactobacillus species, and Klebsiella have been found in ready to eat products (Oranusi and Olorunfemi, 2011).

Food borne diseases are major global problem causing considerable morbidity and mortality annually (Hanson *et al.,* 2012). Bacterial food borne illnesses are among the most wide spread global public health problems of recent times, and their implication for health and economy is increasingly recognized (Addis and Sisay, 2015). Food borne illnesses link to fresh produce are becoming more frequent and wide spread (Warriner *et al.,* 2009). Our food supply has become increasingly globalized, the need to strengthen the food safety is becoming more evident .The presence of various pathogenic bacteria in different foods poses a health hazard and raise concerns about the safety of these food products. Thus, concerns have been raised

by the Food and Agricultural Organization (FAO) and others about these foods serving as a potential source of food poisoning outbreaks (Yeboah-Manu *et al.,* 2010).

Several vegetables such as spinach, cabbage, lettuce and other salad leaves were identified as the commodity group of highest concern from a microbiological safety perspective (Stine *et al.,* 2011; Mritunjay and Kumar, 2015). In developing countries like Nigeria, continued use of untreated waste water and manure as fertilizers for the production of fruits and vegetables contributes so much to contaminations of these products with foodborne pathogens (Amoah *et al.,* 2009; Eni *et al.,* 2010). Therefore, assessing the bacterial contaminant of public health concern is of paramount importance.

2. 0   Materials and Methods
2.1   Source of fruits and vegetables
A total of 30 samples comprising five (5) each of lettuce (*Lactuca sativa)*, cabbage (*Brassica oleracea),* carrot (*Daucus carota)*, apple (*Malus domestica*), guava (*Psidium guajava*) and water melon (*Citrullus lanatus*) were purchased from different vendors in Gombe main market. The samples were placed in clean polythene bags and transported immediately to the laboratory for analysis. Gombe main market is located at Herwagana ward, Gombe Local Government Area of Gombe State, Nigeria.

2.2 Determination of bacteriological loads of the fruits and vegetables
The bacterial loads of the fruits and vegetables were assessed according to Clarence *et al.,* 2009. Specifically, 25g of each sample was washed in 100 ml of sterile distilled water to obtain the first dilution (10^{-1}). Two more dilutions of 10^{-2} and 10^{-3} were derived from the first dilution by using ten (10)-fold serial dilution. An aliquot of 0.1 ml of 10^{1}, 10^{-2} and 10^{-3} dilutions was inoculated on Nutrient Agar and MacConkey Agar plates using pour plate technique. The plates were incubated for 18-24 hours at 37 ^{0}C after which the colonies formed were counted and expressed as colony forming unit per gram of the sample (cfu/g).

2.3 Isolation of bacterial contaminants of the fruits and vegetables

Nutrient agar, MacConkey Agar and Salmonella-Shigella agar were prepared according to Manufacturer's instruction. Twenty-five grams (25 g)

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of each sample was weighed and washed in 100 ml of sterile distilled water. The media were inoculated with 0.1ml of the rinse water using pour Plate Technique. The plates were allowed to solidify, inverted and incubated at 37 ^{0}C for 24 h. After the incubation period, the different culture plates were examined for bacterial growth. Pure culture of individual colonies was prepared by sub-culture on fresh nutrient Agar plates for identification and further studies (Eni *et al.,* 2010).

2.4 Characterization of the bacterial isolates

Following incubation, the bacterial isolates were observed for cultural and morphological characteristics. Discrete colonies were sub-cultured on Nutrient agar, MacCkonkey agar, Salmonella-Shigella agar and Mannitol salt agar to obtain pure cultures. Pure cultures were identified based on Gram staining and a series of biochemical tests which included Kligler Iron Agar (KIA), Oxidase test, Urease test, Citrate utilization test, Indole test, Coagulase test, Catalase test and Motility test (Shu'aibu *et al.,* 2016).

2.5 Statistical analysis
The data was analysed using One-way Analysis of variance (ANOVA)

3.0 Results and Discussion
3.0 Results

3.1 Fruits and Vegetables Contamination Levels

The samples analysed showed a wide range of bacterial contamination between 3.8×10^4 and 1.1×10^5 and between 1.2×10^5 and 8.7×10^4 for total viable bacterial and faecal coliform counts, respectively (Table 1).

Table 1: Viable bacterial and faecal coliform counts of Fruits and Vegetables

S/N	Fruit/ vegetable	VBC (CFU/g)	Log10	FCC (CFU/g)	Log10
1	Apple	3.8×10^4	4.58	3.7×10^4	4.57
2	Guava	3.7×10^4	4.57	2.9×10^4	4.46
3	Water melon	1.1×10^5	5.04	7.0×10^4	4.85
4	Cabbage	1.1×10^5	5.04	8.7×10^4	4. 99
5	Carrot	2.7×10^5	5.43	1.2×10^5	5.08
6	Lettuce	1.7×10^5	5.23	7.0×10^4	4.85

Key: VBC: Viable bacterial count, FCC: Faecal coliform count, CFU=colony forming unit, g=gram

3.2 Occurrence of bacterial contaminants on the fruits and vegetables

Out of the 30 samples of fruits and vegetables analyzed, 28 were found to contain different types of bacteria. Based on Gram staining and biochemical tests, eight species of bacteria were isolated and identified as *Escherichia coli, Salmonella species, Klebsiella species, Enterobacter species., Pseudomonas species, Staphylococcus aureus, Bacillus cereus, and Shigella species*. The *S. aureus* was found to be the most frequently isolated bacterium in fruits with 50% occurrence in Apple, 28.57% in Guava, 33.33% in water melon; followed by *salmonella species* in Apple (25%), Guava (14.29%), water melon (16.67%) and *Bacillus cereus* in Guava and Water melon each with 14.24% while the least isolated bacteria were *Escherichia coli* (42.86%), *Klebsiella species* (25%) and *Enterobacter species* (33.33%) in Guava, Apple and Water melon, respectively (Table 2).

Table 2: Percentage occurrence of bacterial contaminants on fruits

S/N	Bacterial isolates	Apple	Guava	Watermelon
		Occurrence (%)		
1	*S. aureus*	2(50)	2(28.57)	2(33.33)
2	*B. cereus*	Nd	1(14.29)	1(16.67)
3	*Salmonella* spp.	1(25)	1(14.29)	1(16.67)
4	*E. coli*	Nd	3(42.86)	Nd
5	*Klebsiella* spp.	1(25)	Nd	Nd
6	*Enterobacter* spp.	Nd	Nd	2(33.33)
	Total	4(100)	7(100)	6(100)

Key: Nd: not detected

Similarly, Table 3 shows the occurrence of bacterial contaminants isolated and identified from vegetables where *Klebsiella* species appeared to be the most occurring bacterium in cabbage (30%), Carrot (20%), and lettuce (40%) followed by Shigella species in Carrot (13.33%), Cabbage and lettuce each with 10% of occurrence. However, *S. aureus* and *Enterobacter species* were only detected in Carrot and Cabbage. Moreover, *E. coli* and *P. aeruginosa* were only detected in Carrot and Lettuce and then Cabbage and Lettuce respectively while *Salmonella* Species was only detected in Carrot (Table3).

Table 3: Percentage occurrence of bacterial contaminants on vegetables

S/N	Bacterial isolates	Cabbage	Carrot	Lettuce
		Occurrence (%)		
1	*S. aureus*	3(30)	4(26.67)	Nd
2	*Salmonella* spp.	Nd	1(6.67)	Nd

3	*E. coli*	Nd	3(20)	3(30)
4	*Klebsiella spp.*	3(30)	3(20)	4(40)
5	*Pseudomonas* spp.	1(10)	Nd	2(20)
6	*Enterobacter* spp.	2(20)	2(13.33)	Nd
7	*Shigella* spp.	1(10)	2(13.33)	1(10)
	Total	10(100)	15(100)	10(100

Key: Nd: Not detected

4.0 Discussion

The consumption of unsafe foods has been one of the global public health issues in African underdeveloped countries (Shu'aibu *et al.,* 2016). Microbial food contamination constitutes most of the risk of foods unsafety leading to foodborne infections (Shu'aibu *et al.,* 2021).

Fruits and vegetables are widely exposed to microbial contamination which can occur during any step of the farm-to-table due to environmental, animal, and human sources as well as technological applications (Shu'aibu *et al.,* 2021). Therefore, they harbour a diverse range of microorganisms including plant and human pathogens (Carmo *et al.,* 2004; Eni *et al.,* 2010). Fruits and vegetables are commonly consumed raw without further heating process, hence the possibility of food poisoning and food-borne infections always exists (Aycicek *et al.,* 2006; Sarker *et al.,* 2018). The bacterial populations (bacterial load) in this study vary from 3.7×10^4 to 2.7×10^5 and 2.9×10^4 to 8.7×10^4 for aerobic mesophilic and faecal coliform counts, respectively (Table 1). The populations of bacteria in fresh produce could vary widely. Aerobic plate counts could be as high as 10^9 CFU/g but more typically range from 10^4 to 10^6 CFU/g (Nguyen-the and Carli, 1994).

A total of 52 bacteria were isolated from both the fruits and vegetables in this study. On the basis of cultural, morphological and biochemical characteristics, eight species, namely *Escherichia coli, Staphylococcus aureus, Klebsiella* species*, Enterobacter species, Pseudomonas aeruginosa, Bacillus cereus* and *Shigella* species were identified.The bacteria isolated in this study have previously been isolated from fruits and vegetables in other studies both in Nigeria and other countries (Tambaeker and Mundhada, 2006; Eni *et al.,* 2010). Moreover, the bacterial contaminants isolated in this study might be a direct reflection of the sanitary quality of the irrigation water, harvesting, transportation, storage, processing and handling of the produce as suggested by the previous reports (Ray and Bhunia, 2007). Bacteria may be transferred from storage facilities to fruits and vegetables and a cross contamination may occur especially when they are washed with the same wash water by the food vendors or handlers (Eni *et al.,* 2010). More importantly, bacteria on the fruits and vegetables may multiply over time depending on the storage conditions (Montville and Matthews, 2008; Abadias *et al.,* 2008).

Among the bacterial contaminants isolated from the fruits samples, *S. aureus* has higher percentage of occurrence with 50% from apple, 28.57% from guava and 33.33% from water melon followed by *Salmonella* species with 25% from apple 14.29% from guava and 16.67% from water melon, *E. coli* with 42.86% from guava, *Bacillus* species with 14.29% from guava, 16.67% from water melon, *Enterobacter* species with 33.33% from water melon and *Klebsiella* species with 25% from apple (Table 2). On vegetables, *Klebsiella* species has higher percentage of occurrence with 30% from cabbage, 20% from carrot and 40% from lettuce, followed by *S. aureus* with 30.60% from cabbage, and 26.67% from carrot, followed by *E. coli* with 20% from carrot and 30% from lettuce, followed by *Shigella* species with 10% from cabbage, 13.33% from carrot and 10% from lettuce, *Enterobacter* species with 10% from cabbage, and 13.33% from carrot, followed by *P. aeruginosa* with 10% from cabbage and 20% from lettuce, and *salmonella* species with 6.67% from carrot (Table 3). *S. aureus* was found to be the most frequently isolated bacteria from fruits followed by *Salmonella species* and *E. coli*. On vegetables *Klebsiella* species was the most frequently isolated organism followed by *S. aureus.* Viswanathan and Kaur (2001) also reported the presence of *E. coli* and *S. aureus* on fruits and vegetables. Several studies have proved the presence of *S. aureus* on carrot and other ready-to-eat vegetables such as salads (Kaur and Bhowate, 2017).

Staphylococcal species and *E. coli* have been reported to be the major bacteria associated with the contamination of vegetables and fruits (Ibeyessie, 2007). Eni *et al.,* 2010 reported the presence of *Bacillus* species, *Pseudomonas species* and other bacteria while assessing the bacterial contaminants of fruits and vegetables. Some of the bacteria isolated in this study may be part of the natural flora of the fruits and vegetables or contaminants from soil, irrigation water, and environment during transportation, wash water or handlers and vendors (Ofor *et al.,* 2009). The presence of *S. aureus* could be as a result of human contact which indicates possible poor hygiene practices of the handlers since this organism is a normal flora of the skin and nasal passage (Eni *et al.,* 2010). *Pseudomonas* species and *Bacillus species* are part of plant microflora and are among the most common vegetable spoilage bacteria (Ajayi *et al.,* 2017). The presence of *E. coli, Salmonella* species and other *Enterobacteria* is an indication of possible faecal contamination of the fruits and vegetables, water or food processing and poor hygienic practices and

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handling (Tambekar *et al.,* 2007). Therefore, the presence of *E. coli, S. aureus, B. cereus* and other bacteria in this study demonstrates a potential health risk as these organisms are pathogenic and have been implicated in food borne diseases (Granum, 2005; Wagner, 2009; CFIA, 2009). *Spondias mombins* is a plant very reach in important phytochemical constituents and the ethanol extract revealed the presence of some of those pharmacological constituents. The extract was shown to have no obvious physical sign of toxicity and it doesn't cause mortality in all the tested animals. The likely anticancer potential of the extract is similar to that seen in doxorubicin at the tested doses. Ethanol extract of the plant reversed loss in weight, deterioration in liver enzymes, and protein production to an extent similar to that of standard anti-cancer agents.

5.0 Conclusion

The fruits and vegetables investigated in this study have been contaminated with different types of bacterial genera of public health concern. This shows that fruits and vegetables require effective decontamination to avoid outbreak following consumption. Further research to assess the effectiveness of household disinfectants used in the treatment of fruits and vegetables is recommended.

Declarations:
Competing interests
The authors declare that they have no competing interests.
Ethics approval and consent to participate
Not Applicable.
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Consent for publication
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Availability of data and material
Not Applicable.

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