Microbial Assessment of Leafy and Salad Vegetables Sold in Five Different Markets in Lagos State, Nigeria

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Abstract: Vegetables are essential protective food for good health and are also potential reservoir of microbial contaminants. Intake of raw vegetables contaminated with detrimental microorganisms may result in food poisoning. Thus this study assessed the microbial loads of leafy and salad vegetables from five different markets in Lagos State. Macerated samples of the vegetables were analyzed for microbial load using pour plate method. Bacteria were putatively identified on the basis of their phenotypic and biochemical characteristics and fungal isolates were identified by lactophenol-in cotton blue staining. The total viable counts recorded in the vegetables samples ranged from $2.2 x10^4$-14.8×10^4cfu/g, coliform counts show $1.0 x10^4$–5.6×10^4cfu/g, while *Salmonella-Shigella* count ranged from0.2×10^4–8.5×10^4cfu/g, and fungal count ranged from 0.6×10^4 – $7.5 x10^4$cfu/g. A total of 145 bacterial and 26fungal isolates were identified from this study. Among the bacterial isolates, St*aphylococcus* spp(26.21%) were the most predominant bacteria associated with leafy and vegetable salad in the five markets assessed. This was followed by *Micrococcus* spp (13.8%), *Bacillus* spp (12.42%), *Lactobacillus* spp. (8.28%), *Pseudomonas* spp (3.45%) and *E. coli* (3.45%) recorded highest percentage of occurrence for the coliform. Among the fungal isolates, highest percentage of occurrence was recorded for *Saccharomyces* spp (33.33%) followed by *Candida* spp (28.56%), while other bacterial and fungal species were low in their percentage of occurrence. This study showed that vegetables sold in open market are potential source of pathogenic microorganisms and may present health risk to humans when consumed, thus the need for consistent hygienic practices.

Keywords: Vegetables, Microbial load, Open markets, Pathogenic microorganism

INTRODUCTION

The importance of vegetable as a balanced diet cannot be overemphasized; their intake has been reported to lower incidence of cardiovascular disease and obesity (Pem and Jeewon, 2015). Vegetable contain varying content of proteins or fats, supply different proportions of vitamins such as Vitamin A, Vitamin K, and Vitamin B6, pro-vitamins, dietary minerals, carbohydrates and minerals to the diet. They are the sources of phytochemicals that functions as antioxidants, phytoestrogens and anti-inflammatory agents. They are valuable in protective mechanisms like cell maintenance, DNA repair and longevity (Dhellot *et al.*, 2006).

In previous years, this knowledge has steadily resulted in a generalized change of eating habits towards an increasing intake of fruits and vegetables. However, the surfaces of raw vegetables are contaminated with a variety of microorganisms which undermine their nutritional and health benefits, thus increasing the outbreaks of human infections associated with the consumption of fresh or minimally processed fruits and vegetables. Exposure to microbial contamination and microbial growth on vegetables can occur at various stages of harvest (Berger *et al.*, 2010; Seo *et al.*, 2010). Contamination during production occurs due to contact with soil or manure, irrigation water or due to birds and animal faecal matter(Brackett *etal.*,2001).

Bacteria involved in spoilage of vegetables are usually pectinolytic species of the Gram negative genera of *Erwinia*, *Pseudomonas*, *Clostridium*, and *Xanthomonas* and the non-sporing Gram positive organisms like *Corynebacterium* (Uzeh *et al.*, 2009). Certain fungi such *as Aspergillus, Fusarium and Penicillium* species are the commonly occurring filamentous fungi which grow on vegetables and their growth may result in the production of toxins known as mycotoxins which can cause a variety of ill effects in human from allergic response to immunosuppression and cancer (Egbuta *et al.*, 2017).

Consumption of raw vegetables contaminated with harmful microorganisms may result in food poisoning. The escalating number of outbreaks of food poisoning has necessitated the significance of microbiological quality control practices in the food industry. Microbiological risk assessment is an emerging tool for the evaluation of the safety of food and water supplies. Microbiological risk assessment is important for appropriate remedial measures to be embraced to reduce the incidences of food-borne illness due to intake of contaminated foods. Thus, this study was designed to determine the microbial quality of some fresh vegetables sold at five different markets in Lagos Metropolis.

MATERIALS AND METHODS

Sample Collection

A total of 40 samples of leafy and salad vegetables (Ugwu leaf, bitter leaf, scent leaf, water leaf, cabbage, lettuce, spring onions, and spinach) were randomly collected from 5 different open marketsin five local governments in Lagos, Nigeria. These vegetables are displayed in the open at temperature of 35^{o}C. The samples were placed in separate sterile plastic bags kept in an ice box maintained at 6–10°C and transported to the laboratory for microbial analysis within 2–4 hrs.

Enumeration and Isolation of Microorganisms

Twenty five gram (25 g) of each vegetable samples were aseptically weighed and macerated and 225 ml of sterile distilled water was added. Serial dilution of the mixture was carried out using sterile distilled water as diluents. One milliliter dilutions of 10^{-2}, 10^{-4}, 10^{-5}dilution factors were pipetted into sterile Petri-dishes and molten agar (45^{o}C) was added and swirled thoroughly to allow even distribution. Five different types of culture media used were: Nutrient Agar (NA), MacConkey agar(MA), *Salmonella-Shigella* agar (SSA), Potato Dextrose agar(PDA) and Baird-Parker agar(BPA). The media used were weighed and prepared according to the manufacturer's instructions. Plating was done in duplicates, the inoculated Nutrient Agar(NA), MacConkey Agar(MA), Salmonella-Shigella Agar(SSA) and Baird-Parker Agar(BPA) plates were incubated at 37^{o}C for 24-48 h while Potato Dextrose Agar(PDA), plates were incubated at 25^{o}C for 48-72 h. After incubation, colonies that developed on the plates were counted to obtain total viable count. Pure cultures of the isolates were obtained by repeated sub-culturing on fresh agar plates for further studies and identification. For bacterial colony purification, the streak techniques on the Nutrient agar was employed, while the fungal isolates were transplanted to new set of PDA agar by picking.

Identification of the Isolates

Isolates identification was achieved based on their colonial morphology,cellular morphology and biochemical characteristics were characterized according to the taxonomic scheme of Buchanan and Gibbon (1994).

Distinctive morphological properties of each pure culture such as colony form, elevation, margin, color and consistency were observed. The bacterial isolates were also identified by comparing their characteristics with those of known taxa, as described by Jolt *et al*. (1994) and Oyeleke and Manga (2008). The pure isolated fungi were identified using cultural and morphological features according to the most recognized keys in fungal identification (Domsch *et al*., 1993; Klich, 2002; Samson and Varga, 2007).Gram staining and Biochemical tests such as IMViC (Indole, Methyl Red, Voges-Proskauer, and citrate), urease, oxidase, catalase, and triple sugar iron and sugar fermentation were done on the bacterial isolates for proper characterization and identification.

RESULTS

The microbiological results of the sampled vegetables are presented in Table 1. From the results obtained, it was evident that all the vegetables analyzed were contaminated with bacteria. Water leaf was the most contaminated of all the vegetables across the five markets studied and this was closely followed by spinach. The total viable bacteria count recorded ranged from $10.0x10^4$ to $12.0x10^4$ for water leaf and spinach $8.3x10^4$-$9.5x10^4$while spring onion and cabbage recorded the lowest total viable bacteria count of $2.7x10^4$ to 3.9 $x10^4$and 2.2 $x10^4$-$4.7x10^4$ respectively as presented in Table 1.

The total coliform count recorded was also high in some of the vegetable samples and the rate of contamination followed the same pattern as that observed with the total viable bacteria count. Water leaf recorded coliform counts as high as $4.8x10^4$-$5.6x10^4$while spring onion and cabbage recorded lowest level of coliform counts that ranged from 0.9 $x10^4$-$1.5x10^4$and $1.0x10^4$-$1.5x10^4$ respectively. Equally, Staphylococcal count recorded was also high for water leaf with mean count of $4.0x10^4$- $5.5x10^4$while spring onion, cabbage and lettuce recorded the lowest Staphylococcal count of $0.7x10^4$-$1.7x10^4$as shown in Table 1. Water leaf showed the highest *Salmonella-Shigella* count that ranged between $1.7x10^4$ to 8.5 $x10^4$ and this was closely followed with spinach having $1.1x10^4$ to $1.7x10^4$while no count was recorded for cabbage, lettuce and spring onion.

Likewise, fungal count was found to be high in most of the vegetables analyzed, fungal count as high as $7.5x10^4$was recorded in one of the sample of lettuce examined. The fungal count recorded for water leaf was lower when compare to the bacteria counts that were recorded for the water leaf. Cabbage had the lowest fungal count that ranged from $0.5x10^4$to $0.8x10^{4.}$

Based on the cultural, morphological and biochemical characteristics of the bacteria isolated from the vegetable samples, four different phyla(Firmicutes, Proteobacteria, Actinobacteria and Bacteroidetes) comprising of 23 different genera were identified as shown in Table 2.Most of the isolates belong to the phylum Proteobacteria and this is closely followed by the Firmicutes group. *Staphylococcus* spp and *Micrococcus* spp were isolated in all the five markets studied.

Table 1: The Microbial Population of Leafy and Salad Vegetables

Sample Name	Vegetable Code	Total Viable Count on Nutrient Agar (Cfu/g)	Coliform count on MacConkey agar (Cfu/g)	Staphylococcus count on Baird parker agar (cfu/g)	Salmonella Shigella count on Salmonella Shigella agar (Cfu/g)	Fungal count on Potato dextrose agar (Cfu/g)
A	BL	6.2X10^4	2.4X10^4	2.7X10^4	0.0X10^4	1.3X10^4
	WL	14.8X10^4	4.8X10^4	5.5X10^4	2.0X10^4	1.6X10^4
	LS	9.5X10^4	4.0X10^4	3.4X10^4	1.7X10^4	1.2X10^4
	PL	8.2X10^4	3.1X10^4	2.3X10^4	0.9X10$^{4`}$	2.1X10^4
	SL	8.1X10^4	2.7X10^4	3.1X10^4	0.7X10$^{4`````}$	2.0X10^4
	CAB	3.0X10^4	1.5X10^4	1.1X10^4	0.0X10^4	0.6X10^4
	LET	4.1X10^4	2.1X10^4	1.6X10^4	0.0X10^4	0.9X10^4
	SPR.ON	2.7X10^4	0.9X10^4	0.7X10^4	0.0X10^4	0.7X10^{4s}
B	BL	8.6X10^4	2.6X10^4	3.8X10^4	0.5X10^4	2.0X10^4
	WL	12.5X10^4	5.6X10^4	4.9X10^4	8.5X10^4	1.6X10^4
	LS	9.3X10^4	4.4X10^4	3.7X10^4	1.2X10^4	2.5X10^4
	PL	7.8X10^4	2.6X10^4	2.6X10^4	0.6X10^4	1.1X10^4
	SL	8.7X10^4	3.6X10^4	3.6X10^4	1.1X10^4	1.7X10^4
	CAB	2.6X10^4	1.1X10^4	1.3X10^4	0.0X10^4	0.8X10^4
	LET	3.7X10^4	1.5X10^4	1.7X10^4	0.0X10^4	1.1X10^4
	SPR.ON	3.5X10^4	1.5X10^4	1.1X10^4	0.0X10^4	1.2X10^4
C	BL	7.1X10^4	2.2X10^4	2.6X10^4	0.2X10^4	2.6X10^4
	WL	11.0X10^4	5.1X10^4	5.1X10^4	4.4X10^4	1.4X10^4
	LS	8.4X10^4	3.6X10^4	1.6X10^4	1.1X10^4	2.4X10^4
	PL	7.3X10^4	3.1X10^4	2.1X10^4	0.8X10^4	2.8X10^4
	SL	8.2X10^4	3.4X10^4	2.5X10^4	0.6X10^4	1.0X10^4
	CAB	4.7X10^4	1.0X10^4	0.9X10^4	0.0X10^4	0.5X10^4
	LET	4.0X10^4	1.7X10^4	1.5X10^4	0.0X10^4	0.9X10^4
	SPR.ON	3.9X10^4	1.3X10^4	1.5 X10^4	0.0x10^4	1.3x10^4
D	BL	6.3x10^4	2.5x10^4	2.8x10^4	0.8x10^4	1.6x10^4
	WL	10.2x10^4	4.9x10^4	4.0x10^4	1.7x10^4	1.7x10^4
	LS	8.3x10^4	3.8x10^4	4.0x10^4	1.5x10^4	2.2x10^4
	PL	7.2x10^4	3.2x10^4	2.1x10^4	1.0x10^4	2.1x10^4
	SL	8.3x10^4	3.4x10^4	2.8x10^4	0.9x10^4	1.6x10^4
	CAB	2.7x10^4	1.2x10^4	1.2x10^4	0.0x10^4	0.8x10^4
	LET	3.8x10^4	2.0x10^4	1.0x10$^{4``}$	0.0x10^4	0.7x10^4
	SPR.ON	3.7x10^4	1.4x10^4	1.0x10^4	0.0x10^4	1.3x10^4
E	BL	6.9x10^4	2.3x10^4	3.3x10^4	0.6x10^4	1.9x10^4
	WL	10.0x10^4	5.0x10^4	4.2x10^4	1.1x10^4	1.5x10^4
	LS	8.9x10^4	4.0x10^4	3.3x10^4	1.4x10^4	2.0x10^4
	PL	7.0x10^4	2.8x10^4	2.2x10^4	0.7x10^4	2.5x10^4
	SL	8.2x10^4	3.2x10^4	2.9x10^4	0.4x10^4	1.9x10^4
	CAB	2.2x10^4	1.4x10^4	1.2x10^4	0.0x10^4	0.8x10^4
	LET	3.4x10^4	1.6x10^4	1.6x10^4	0.0x10^4	7.5x10^4
	SPR.ON	3.3x10^4	1.1x10^4	1.3x10^4	0.0x10^4	0.9x10^4

KEY:
Sample name: A- Agege B- Iyana-Ipaja C- Iyana-Iba D- Oshodi E- Igando
Vegetable code: BL- Bitter leaf, WL - Water leaf, PL – Pumpkin leaf, CAB – CabbageLET-Lettuce, SPR.ON: Spring Onions, SPI: Spinach, SL: Scent leaf, LS is spinach, PL is Ugwu leaf.

Table 2: The Occurrence Of Bacterial Isolates Across The Various Markets.

S/No	Organisms	Agege	Iyana-iba	Igando	Oshodi	Iyana-Ipaja
1	*Bacillus cereus*	+	-	+	+	-
2	*Staphylococcus aureus*	+	+	+	+	+
3	*Escherichia coli*	+	-	-	+	+
4	*Staphylococcus albus*	+	+	+	+	+
5	*Streptococcus faecium*	-	+	-	-	-
6	*Pseudomonas aeruginosa*	+	-	-	+	+
7	*Citrobacterkoseri*	+	+	-	-	-
8	*Enterobacter cloacae*	-	+	-	-	-
9	*Klebsiella pneumonia*	-	+	-	-	-
10	*Flavobacteriumrigense*	+	+	-	-	-
11	*Aeromonnashydrophila*	-	+	-	-	-
12	*Erwiniacarotovora*	-	+	-	-	-
13	*Salmonella* spp.	-	+	-	-	-
14	*Alcaligenesfaecalis*	+	+	-	-	+
15	*Xanthomonas*spp.	+	+	-	-	-
16	*Micrococcus luteus*	+	+	+	+	+
17	*Serratia*spp.	+	+	-	-	-
18	*Bacillus subtilis*	+	+	+	+	-
19	*Clostridium* spp.	+	+	-	-	-
20	*Micrococcus roseus*	+	-	+	+	+
21	*Corynebacterium*spp.	+	+	-	-	-
22	*Erwiniauredovora*	+	+	-	-	-
23	*Bacillus licheniformis*	-	+	-	-	-
24	*Erwiniaamylovora*	-	+	-	-	-
25	*Staphylococcus epidermidis*	+	+	-	-	+
26	*Bacillus brevis*	+	+	-	-	+
27	*Lactobacillus fermentum*	+	-	+	+	+
28	*Micrococcus varians*	+	-	+	+	+
29	*Pseudomonas* spp.	+	+	-	-	-
30	*Klebsiellaliquefaciens*	+	+	-	-	-
31	*Lactobacillus plantarum*	+	+	+	+	+
32	*Acinetobacter*spp.	+	+	-	-	-
33	*Pediococcusdamnosus*	+	+	-	-	+
34	*Staphylococcus hominis*	+	+	-	-	+
35	*Citrobacterdiversus*	+	+	-	-	-
36	*Alcaligenes*spp.	+	+	-	-	+
37	*Lactococcuslactis*	+	+	-	-	+
38	*Enterobacterintermedius*	-	+	-	-	-
39	*Flavobacterium*spp.	-	+	-	-	-
40	*Acetobacter*spp.	-	+	-	-	-

Key{+} = Present {-} = Absent

Table 3: The Occurrence of Fungal Isolates Across The Various Markets

S/No	Organisms	Agege	Iyana-iba	Igando	Oshodi	Iyana ipaja
1	*Saccharomyces cerevisiae*	+	+	+	+	+
2	*Kluyveromycesfragilis*	+	+	-	-	+
3	*Hansenula*spp.	+	+	-	-	+
4	*Candida* spp.	+	+	-	+	+
5	*Geotrichumcandidium*	+	-	+	+	-
6	*Rhodotorulagraminis*	+	-	+	+	-
7	*Geotrichumcapitatum*	+	-	+	+	-
8	*Saccharomyces exigus*	+	-	+	+	-
9	*Rhodotorularubra*	-	-	-	+	-
10	*Saccharomyces rouxii*	+	+	-	-	-
11	*Pichia farinose*	+	+	-	-	-
12	*Candida krusei*	+	+	-	-	-
13	*Candida tropicalis*	+	+	-	-	-
14	*Candida valida*	+	-	+	+	-

Key: {+} = Present, {-} = Absent

DISCUSSION

Vegetables are often consumed by most communities due to their satisfactory quality and rich mineral contents. However, gastroenteritis has been a major effect of consumption of vegetables in developing countries. Also, there have been numerous concerns over the handling and sanitary quality of vegetables thereby exposing them to possible microbial contaminations that poses health risks to consumers. This study revealed the microbial quality of selected vegetable products sold in Lagos.

All the vegetables analyzed were all contaminated with significant numbers of microorganisms. This is similar to the works of Kemajou*et al*. (2017) that recorded bacterial contamination in all five groups of vegetables gotten from Rivers State, Nigeria. It also substantiated the works of Adebayo-Tayo*et al*., (2012) and Eni *et al*., (2010) all of whom reported bacterial contamination to all types of vegetables gotten from Uyo and Sango market in Nigeria. This high level of microbial contamination is asa result of variations in actors such as storage conditions, handling and sanitary qualities, transportation, use of unclean water for washing and most significantly the frequent exposure to the contaminated markets air mostly associated with developing countries.

For this study, water leaves recorded the highest bacterial and coliform counts. This is also seen in the works of Kemajou *et al*. (2017), who recorded the largest bacterial count for water leaves as compared to other vegetables. Also, water leaves have the highest staphylococcal and fungal count across all five markets. This is due to the high moisture content of water leaves, which provide an ideal condition for microorganisms to thrive. Also, the mode of cultivation of the vegetable on humid and contaminated soil account for the high presence of microorganisms on the vegetable.

Forty bacterial isolates and fourteen fungal isolates were identified from the vegetables. Of all the isolates, *Staphylococcus aureus*, *Staphylococcus albus, Micrococcus luteus* and *Lactobacillus plantarum* were found across all the four market sources. This is in tandem with previous studies (Uzeh*et al*., 2009; Goja*et al*., 2013) that also isolated those genera from market vegetables. Also, enteric organisms were also isolated from the vegetables. Peculiarly, *Pseudomonas aeruginosa* and *Escherichia coli* were the leading enteric isolated from the vegetables which have been substantiated with the report of ICSMF (1998), that microorganisms most commonly found in vegetables generally involve *Pseudomonas* and *Erwinia* as coliforms. This has been further proven in the works of Adebayo-Tayo*et al*. (2012) and Kemajou*et al*. (2017) where *Pseudomonas* and *Erwinia sp* are the common enteric organisms found on vegetables sold in the market.

The presence of *Staphylococcus sp* suggests that the sold vegetables are detrimental for human health and unfit for consumption. Most strains of *Staphylococcus*produces heat-stable enterotoxin and they are pathogenic in nature (Marta, 2011). *Erwinia sp*are often associated with plants rot and wilt diseases, thus, the presence of *Erwinia* sp in the vegetables suggests why vegetables spoil quickly in market areas. Most significantly is the presence *of Escherichia coli, Enterobacter sp* and *Salmonella sp* in the vegetable which is an indication of faecal contamination of the food due to unhygienic handling of the produce.

The isolation of major Gram-positive bacteria such as the *Bacillus sp* which is also reported by Kemajou *et al*., (2017) is as a result of the abundance of such microbes in the soil where those plants were cultivated. It is however important to note that some of the bacteria isolated may be part of natural floral of the vegetables (Ofor *et al*., 2009).

Among the fungi isolated from the vegetables*, Saccharomyces cerevisiae* was the most common fungus that was prominent in all vegetable types across the four markets. This is in contrast to the previous works of Adebayo-Tayo*et al*., (2012) and Akintobi *et al*., (2011), where *Aspergillus* sp were the most prominent fungal contamination in vegetables stored in Uyo metropolis, Akwa Ibom, Nigeria. The presence of fungal pathogens such as *Candida* sp also makes the vegetables unfit for consumption.

Another insight gotten from this study is that, of all the four markets, Agege market has higher occurrence of the entire isolated bacterial and fungal species. This is closely followed by Iyana-Iba market which also showed significant presence of those pathogens on the sold vegetables. This suggests that there is high level of unhygienic practices in those markets that local buyers are exposed to more health risk associated with food contamination than others.

Generally, microbial contaminations of vegetables are associated with poor handling practices in storage conditions and food supply chain (Akinmusire, 2011; Akintobi *et al*., 2011). Also, poor transportation of harvested vegetables from rural areas to urban market is also a major channel for microbial contamination of the produce (Baiyewu *et al*., 2007). The non-separation of bruised vegetables from fresh ones also cause cross-infections of the produce sold in various markets (Akintobi *et al*., 2011). All of these poor practices portend great health risk to consumers of these agricultural produce.

CONCLUSION

The study of microbial assessment of food products is sacrosanct as it gives an invaluable insight into the array of microbes that might be implicated with health hazards to consumers. In this study, there were bacterial and fungal contamination in all vegetable types sold in four main markets in Lagos, Nigeria. This contamination is often associated with unhygienic practices in the markets, improper farming conditions, poor transportation etc. The study revealed the presence of forty bacterial and fourteen fungal organisms thereby proving that vegetables can adequately serve as reservoir of various microbes which subsequently might infect a susceptible host. As such, there is an urgent need to encourage the best ideals of farming, processing, retailing and consumption of vegetables to prevent microbial contamination in the least possible way. In addition, Vegetables should be thoroughly washed with safe water or saline solutions before processing and consumption especially where they are not going to be heated or cooked before consumption.

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