Bacteriological quality of street-vended Ready-to-eat fresh salad vegetables sold in Port Harcourt Metropolis, Nigeria

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ABSTRACT: Vegetables promote good health but harbor a wide range of microbial contaminants. To assess the microbial quality of street-vended ready-to-eat fresh vegetables, fourteen samples of cabbage and lettuce vegetable were purchased from different markets. Samples of salad vegetables were analyzed using standard bacteriological methods. The bacteria loads as reflected by the total aerobic count ranged from 3.1 x 10^5 to 7.8 x 10^5CFU/g for cabbage and 3.1 x 10^5 to 6.9 x 10^5 CFU/g for lettuce. The total coliform counts ranged from 3.4 x 10^5 to 5.6 x 10^5 CFU/g for cabbage and 3.4 x 10^5 to 4.0 x 10^5 CFU/g for lettuce. The total Salmonella-Shigella counts ranged from no significant growth (0.0 x 10^5) to 3.6 x 10^5 CFU/g for cabbage and no significant growth (0.0 x 10^5) to 3.4 x 10^5 CFU/g for lettuce. A total number of twelve genera of bacteria were isolated and identified as *Staphylococcus* (7.6%), *Proteus* spp. (5.1%), *Bacillus* spp. (3.4%), *Shigella* spp. (2.5%), *Micrococcus* spp. (1.7%), *Pseudomonas* spp. (7.6%), *Enterobacter* spp. (1.7%), *Serratia* spp. (1.7%), *Citrobacter* spp. (2.5%) *Klebsiella* spp. (6.8%), *Salmonella* spp. (13.6%) and *Escherichia coli* (45.8%). This showed that *Escherichia coli* (45.8%) were most predominant, followed by *Salmonella* spp. (13.6%) while *Micrococcus* spp. (1.7%), *Enterobacter* spp. (1.7%) and *Serratia* spp. (1.7%) were least predominant. Since the vegetables are ready-to-eat and will not be subjected to heat treatment, it could be a source of food poisoning to consumers. However, regular inspections of food premises and education of food vendors has been recognised as one of the measures to ensure improvement of the quality of street foods. Thus, government should placed emphasis on educating vendors on simple preventive steps of keeping food hygienically safe.

[Odu Ngozi Nma and Okomuda Mary Oruese. **Bacteriological quality of street-vended Ready-to-eat fresh salad vegetables sold in Port Harcourt Metropolis, Nigeria.** *Academia Arena*, 2013,5(3):65-75] (ISSN: 1553-9865) http://www.sciencepub.net/academia. 9

Keywords: Fresh vegetables, Salads, Ready-to-eat, Street-vended, Pathogens, Public health

1. INTRODUCTION

Ready-to-eat (RTE) salads constitute an expanding food commodity nowadays served to these consumers (Carrasco *et al*., 2010; Arvanitoyannis *et al*., 2011; Adjrah et al., 2013). Salad can be defined as a food made primarily of a mixture of raw vegetables and/or fruits (Uzeh *et al*., 2009; Rajvanshi, 2010; Adjrah et al., 2013). Vegetables can also be regarded as the edible component of plants, such components includes leaves, stalk, roots, tubers, bulbs, flowers and seed (ICMSF, 1998; Adebayo-Tayo et al., 2012). These include those plants or plant part used in making soup or served as an integral part of main meal (Yusuf et al., 2004). Vegetables are important protective food and highly beneficial for the maintenance of health and prevention of diseases (Adebayo-Tayo et al., 2012). They contain valuable food ingredients which are essential for the proper function of the body. Vegetable contain various medicinal and therapeutic agent and are valued mainly for their high vitamin and mineral content (Adebayo-Tayo et al., 2012). Studies have evaluated the association of fruit and vegetables consumption with the reduction of risk of specific diseases (Hung et al., 2004; Adebayo-Tayo et al., 2012).

Fruits and vegetables carry microbial flora while passing from the farm to the table. The produce is exposed to potential microbial contamination at every steps including cultivation, harvesting, transporting, packaging, storage and selling to the final consumers, which may affect the health of consumers. Possible sources of these pathogens are soil, faeces, manure (both of human and animal origin), water (irrigation, cleaning), animals (including insects and birds), handling of the products harvesting and processing equipment, and transportation (Okonko *et al*., 2008a,b,c,d,). Most of raw vegetables such as cabbage and lettuce are normally consumed without being cooked, so the possibility of food poisoning exists (Aycicek *et al*., 2006). Unfortunately, the increase in consumption has been correlated with the increase frequency of outbreak of illness associated with the raw fruits and vegetable. Previous investigations have shown that efficacy of washing and sanitizing treatment reduces microbial populations on fresh vegetables.

It is clearly evident that a large number of vegetables are a good source of antioxidants and phytonutrients, and have health protecting properties (Meng *et al*., 2002; Heo and Lee, 2006; Vrchovska *et al*., 2006; Adjrah et al., 2013), to improve human well being. In contrast with these advantages, the salads containing raw vegetables may be unsafe, mainly because of the environment under which they are prepared and consumed (Taban and Halkman, 2011; Adjrah et al., 2013) and also of the lake of personal hygiene (Martinez-Tomé *et al*., 2000; Cuprasitrut *et al*., 2011; Adjrah et al., 2013). These foods have been identified as vehicles of bacterial agents and generate food safety problems, especially gastroenteritis (Meng *et al*., 2002; Adu-Gyamfi and Nketsia-Tabiri, 2007; Adjrah et al., 2013). The incriminated microorganisms included *Pseudomonas* spp., *Xanthomonas* spp., *Enterobacter* spp., *Chromobacterium* spp., lactic acid bacteria, yeasts, less frequently *Aeromonas hydrophila*, and occasionally *Listeria monocytogenes* (Lavelli *et al*., 2006; Adjrah et al., 2013).

Frequent food borne diseases have been reported in Nigeria following the consumption of vegetables. Vegetable salads do not need to be heated before consumption, whereas, vegetables may act as a reservoir for many microorganisms (Beuchat, 2002). Therefore, RTE salads vegetables carry the potential risk of microbiological contamination due to the usage of untreated irrigation water or sewage, inappropriate organic fertilizers or inadequately composted manure, the harvesting, the handling, processing and distributing during the restaurant services (Taban and Halkman, 2011; Adjrah et al., 2013). Previous research pointed out that vegetables produced in Lomé represent a microbiological risk for consumers (Adjrah *et al*., 2011, 2013). The present study evaluates the bacteriological quality of street-vended ready-to-eat fresh salad vegetables commonly consumed in Port Harcourt metropolis, Rivers State, Nigeria.

2. MATERIALS AND METHODS

2.1. Collection and Processing of samples

A total of 14 randomly selected healthy and clean looking fresh salad vegetables were purchased from Choba market, Rumukoro market, Mile 3 market, Creek road and Alakahia market all in Port Harcourt, Rivers State, Nigeria. These samples were placed in separate sterile plastic bags and transported to the laboratory for bacteriological analysis. These vegetables were not locally cultivated in the state. Vegetables were surface sterilized by exposing them in 1 min 90% ethyl alcohol (BDH chemicals Ltd Poole England) and then 3 min to 1% sodium hypochlorite and then rinsed three times in sterile distilled water. Segments (3 - 5 cm) of tissues from the margins of the vegetables were cut out with a sterile scalpel and placed on previously prepared media in Petri dishes and incubated at appropriate temperatures.

2.2. Enumeration, Isolation and Identification of Bacteria Isolates

The vegetable samples from different location were weighed and grinded using stomacher. Twenty-five grams of each homogenized sample was dispensed into a prepared 225 ml of normal saline. The content was shaken for homogenous mixture. Ten fold serial dilutions were used to prepare culture plates by pour plate method. About 0.1 ml of the 10^{-5} dilution of the samples from different location were pipetted out and pour plated using Plate Count agar (PCA), Nutrient agar (NA), MacConkey agar (MCA), Eosin Methylene Blue Agar (EMB) and Salmonella-Shigella agar (SSA) for total aerobic counts, total coliform counts and total Salmonella-Shigella counts. These plates were incubated at 37^0C for 24-48 hours. The streak technique in the Nutrient agar was employed for bacterial colony purification. The discrete colonies from these subcultured plates and series of biochemical tests were done for proper characterization and identification. 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).

3. RESULTS ANALYSIS

The results obtained for microorganisms associated with the street-vended ready-to-eat fresh salad vegetables are shown in Tables 1 to 2.

3.1. Enumeration of Isolates

Table 1 showed that the total viable counts for cabbage ranged from 3.1 x 10^5 to 7.8 x 10^5 CFU/g. It also showed that the total viable counts for lettuce ranged from 3.1 x 10^5 to 6.9 x 10^5 CFU/g (Table 1). Table 1 also shows the total coliform counts for vegetables (cabbage and lettuce). It showed that the total coliform counts ranged from 3.4 x 10^5 to 5.6 x 10^5 CFU/g for cabbage and 3.4 x 10^5 to 4.0 x 10^5 CFU/g for lettuce (Table 1). The total Salmonella-Shigella counts ranged from no significant growth (0.0 x 10^5) to 3.6 x 10^5 CFU/g for cabbage and no significant growth (0.0 x 10^5) to 3.4 x 10^5 CFU/g for lettuce. For cabbage samples, no significant growth of Salmonella-Shigella in samples C, D, E and F while for lettuce, no significant growth of Salmonella-Shigella was seen in samples C, D, F and G. No significant growth of coliforms was found in samples D and F of cabbage (Table 1).

Table 1: Microbial Loads of Isolates obtained from Vegetables

Samples	Type	Total Viable Counts (CFU/g)	Total Coliform Counts (CFU/g)	Total Salmonella-Shigella Counts (CFU/g)
A	Cabbage	6.6×10^5	4.2×10^5	3.2×10^5
B	Cabbage	7.8×10^5	5.6×10^5	3.6×10^5
C	Cabbage	3.1×10^5	4.8×10^5	NSG
D	Cabbage	5.8×10^5	3.4×10^5	NSG
E	Cabbage	6.9×10^5	3.9×10^5	NSG
F	Cabbage	5.7×10^5	4.0×10^5	NSG
G	Cabbage	7.3×10^5	3.4×10^5	3.1×10^5
A	Lettuce	6.6×10^5	4.0×10^5	3.4×10^5
B	Lettuce	3.1×10^5	3.6×10^5	3.4×10^5
C	Lettuce	5.4×10^5	4.0×10^5	NSG
D	Lettuce	5.2×10^5	NSG	NSG
E	Lettuce	6.4×10^5	3.8×10^5	3.2×10^5
F	Lettuce	4.7×10^5	NSG	NSG
G	Lettuce	6.9×10^5	3.4×10^5	NSG

Key: NSG- No Significant Growth

3.2. Isolation and Identification of Isolates

The bacteria isolated from vegetables were identified based on their cultural, morphological and biochemical characteristics as *Staphylococcus* spp., *Proteus* spp., *Bacillus* spp., *Shigella* spp., *Micrococcus* spp., *Pseudomonas* spp., *Enterobacte*r spp., *Serratia* spp., *Citrobacter* spp., *Klebsiella* spp., *Salmonella* spp. and *Escherichia coli*.

3.3. Frequency of occurrence of Isolates

Table 2 shows the frequency of occurrence of bacteria associated with vegetables in Port Harcourt metropolis, Nigeria. It showed that *Escherichia coli* (45.8%) were most predominant bacterial isolates associated with vegetable samples used in this study. This was followed by *Salmonella* spp. (13.6%). *Enterobacter* spp. (1.7%), *Serratia* spp. (1.7%) and *Micrococcus* spp. (1.7%) were least predominant (Table 2). *Escherichia coli* were present in all the vegetables examined. *Enterobacter* spp., *Serratia* spp., *Micrococcus* spp., *Pseudomonas* spp., and *Citrobacter* spp. were only present in lettuce while *Bacillus* spp. was only present in cabbage (Table 2).

Table 2: Frequency of occurrence of Bacteria Associated with Vegetables in Port Harcourt Metropolis, Nigeria

Isolates	No. (%)	Cabbage (%)	Lettuce (%)
Escherichia coli	54(45.8)	43(79.6)	11(20.4)
Proteus spp.	6(5.1)	4(66.7)	2(33.3)
Bacillus spp.	4(3.4)	4(100.0)	0(00.0)
Staphylococcus aureus	9(7.6)	5(55.5)	4(44.5)
Enterobacter spp.	2(1.7)	0(00.0)	2(100.0)
Salmonella spp.	16(13.6)	8(50.0)	8(50.0)
Serratia spp.	2(1.7)	0(00.0)	2(100.0)
Klebsiella spp.	8(6.8)	6(75.0)	2(25.0)
Micrococcus spp.	2(1.7)	0(00.0)	2(100.0)
Pseudomonas spp.	9(7.6)	0(00.0)	9(100.0)
Citrobacter spp.	3(2.5)	0(00.0)	3(100.0)
Shigella spp.	3(2.5)	2(66.7)	1(33.3)
Total	**118(100.0)**	**71(60.7)**	**46(39.3)**

4. DISCUSSION

The present study evaluated the bacteriological quality of street-vended ready-to-eat fresh salad vegetables commonly consumed in Port Harcourt metropolis, Rivers State, Nigeria. The result showed that that *E. coli* was most predominant (45.8%), followed by *Salmonella* spp. (13.6%) while *Micrococcus* spp. (1.7%), *Enterobacter* spp. (1.7%) and *Serratia* spp. (1.7%) were least predominant bacteria isolated from the salad vegetables examined. The incidence of bacteria in salad vegetables may be expected to reflect the sanitary quality of the processing steps and the microbiological condition of the raw product at the time of processing (Ngugen, 1994; Adebayo-Tayo et al., 2012). For almost 100 years, vegetables contaminated in the field have been recognized as a source of human infection and unless. Many of the viruses (Rosenblum et al., 1990), bacteria (Ho et al., 1986) and protozoan on vegetables which have caused food poisoning are derived from human faeces (Adebayo-Tayo et al., 2012). However, pathogenic microorganism of human origin may also be present in minimally processed vegetables as the minimal technological processing may be unable to remove the original contamination resulting from air, soil, water, insects, animals, workers, harvesting and transportation equipment (Adebayo-Tayo et al., 2012).

Bacteria most commonly found in vegetables generally involve *Pseudomonas* spp. and *Erwinia* spp. as coliforms and *Micrococcus* spp. (ICSMF, 1998). In this study, the bacteria isolated from vegetables were *Staphylococcus* (7.6%), *Proteus* spp. (5.1%), *Bacillus* spp. (3.4%), *Shigella* spp. (2.5%), *Micrococcus* spp. (1.7%), *Pseudomonas* spp. (7.6%), *Enterobacter* spp. (1.7%), *Serratia* spp. (1.7%), *Citrobacter* spp. (2.5%) *Klebsiella* spp. (6.8%), *Salmonella* spp. (13.6%) and *Escherichia coli* (45.8%). All the bacteria reported in this study had previously been isolated from vegetables in other studies, both in Nigeria and elsewhere (Dunn et al., 1995; Adebolu and Ifesan, 2001; Omemu and bankole, 2005; Tambekar and Mundhada, 2006; Uzeh et al., 2009; Yeboah-Manu et al., 2010; Rajvanshi, 2010; Halablab et al., 2011; Ameko et al., 2012; Odu and Akano, 2012; Adjrah et al., 2013). Halablab et al. (2011) reported that lettuce samples from the Bekaa Valley in Lebanon had *E. coli, S. aureus* and coliforms. In Rajvanshi (2010), all the samples of street vended salads in Jaipur City, India, carried gram positive as well as gram negative bacteria; and samples contaminated with certain pathogens were *Bacillus* (24.5%), *E. coli* (11.8%), *Pseudomonas* (11.8%), *Staphylococcus* (10.9%), *Enterobacter* (9.0%), *Streptococcus* (6.4%), *Klebsiella* (5.4%) and *Citrobacter* (3.6%) in their study (Rajvanshi, 2010; Ameko et al., 2012). Pathogens identified by Yeboah-Manu et al. (2010) in their study were *E. coli, P. aeruginosa, K. pneumoniae*, and *Streptococcus* sp., but *S. aureus* was absent. In the Odu and Akano (2012) study on shawarma, *Proteus* spp. (22.7%) was the most predominant, followed by *Escherichia coli* (13.6%), *Bacillus* spp. (13.6%) and *Staphylococcus aureus* (13.6%). *Enterobacter aerogens* (9.1%), *Klebsiella* spp. (9.1%), *Serratia marcescens* (9.1%), and *Micrococcus* spp. (9.1%) were least predominant.

Pathogens such as *Bacillus cereus, Salmonella* and *Escherichia coli* are naturally present in some soil, and their presence on fresh vegetables is not rare (Odu and Akano, 2012; Adebayo-Tayo et al., 2012). Sufficient moisture, abusive temperature and adequate time will ensure a continuing increase in the bacteria population (Adebayo-Tayo et al., 2012). In the study by Adjrah et al. (2013), *Salmonella* spp. was not detected in any of the samples evaluated, but almost 25.0% of the samples were contaminated by *S. aureus*. In their study (Adjrah et al., 2013), the percentage of samples positive for indicator of food safety lack germs like total aerobic bacteria, total coliforms and thermotolerant coliforms were 100, 100 and 37.68 respectively; corresponding to conformity rates of 14.49, 11.59 and 81.16 respectively. In a study by Rajvanshi (2010), the percentage of salads vended on Jaipur City Street in India which were colonized by *S. aureus* was below 10.9%. In contrast, Feglo and Sakyi (2012) did not isolate *S. aureus* in any sample of salads collected in Kumasi (Ghana).

Cenci-Goga *et al*. (2005) pointed out that total aerobic bacteria count was a good indicator of food safety. Bacterial counts on street-vended ready-to-eat cabbage and lettuce exceeded the recommended World Health Organization (WHO, 1996) and International Commission on Microbiological Specifications for Food (ICMSF, 1998) standards of 10^3 CFU/g (for example, Log10 3.0 CFU/g) (Amponsah-Doku et al., 2010; Ameko et al., 2012). This agrees with the results obtained in this study, where bacterial counts on all salad vegetable (cabbage and lettuce) samples exceeded Log10 3.0 CFU/g. In this study, total viable counts were 3.1×10^5 to 7.8×10^5 CFU/g for cabbage and 3.1×10^5 to 6.9×10^5 CFU/g for lettuce. A similar study was carried out in Lagos by Uzeh *et al*. (2009) and the total aerobic bacteria count ranged from 3.3×10^3 to 5.9×10^6 CFU/g. Also, a previous study carried out by Odu and Akano (2012) shawarma showed higher TVC for aerobic mesophillic bacteria for all 3 locations and the home made samples used in their study. The total aerobic bacteria count in the Odu and Akano (2012) study were 1.1×10^6CFU/g, 8.0×10^5CFU/g, 9.0×10^5CFU/g and 4.2×10^3CFU/g respectively, with Elelenwo and GRA having the highest TVC, while the total viable bacterial count for both Choba and home-made samples were the lowest ($8.0x10^5$ and $4.2x10^3$ CFU/g).

In this study, total coliform counts were 3.4 x 10^5 to 5.6 x 10^5 CFU/g for cabbage and 3.4 x 10^5 to 4.0 x 10^5 CFU/g for lettuce. The same findings were reported by Hanashiro *et al.* (2005) in São Paulo for thermotholerant coliforms load. Total Salmonella-Shigella counts were 0.0 x 10^5 to 3.6 x 10^5 CFU/g for cabbage and 0.0 x 10^5 to 3.4 x 10^5 CFU/g for lettuce. The higher bacterial count observed for the vegetables examined in this study are similar to those obtained in other studies in Nigeria (Uzeh et al., 2009; Bukar et al., 2010; Adebayo-Tayo et al., 2012; Ameko et al., 2012; Odu and Akano, 2012). The total aerobic counts obtained in this study is lower compared to that reported by Kaneko et al. (2003) and Adebayo-Tayo et al. (2012), however, the total coliform counts were slightly higher than that reported by Kaneko et al. (2003), Odu and Akano (2012), and Adebayo-Tayo et al. (2012). Results of the microbiological analysis of raw mixed vegetable salads by Ameko et al. (2012) indicate that 20% of the vendors had the salads that they sold in the mornings with microbial loads in excess of 5×10^4 cfu/g, and this increased to 80% of the vendors in the afternoons. According to Amponsah-Doku et al. (2010), thermotolerant coliforms on lettuce varied from 2.3×10^3 to 9.3×10^8 CFU/g on farm, 6.0×10^1 to 2.3×10^8 CFU/g on market and 2.3×10^6 to 2.4×10^9 CFU/g at street-food vendor sites in Kumasi.

The results of this present study agree with those of Adu-Gyamfi and Nketsia-Tabiri (2007) and Ameko et al. (2012), where samples of vegetable salads, served with waakye, had higher levels of contamination. Aerobic mesophyllic counts of 6.9 and 7.6 and coliforms counts of 5.7 and 6.4 log10 cfu/g, were obtained by Adu-Gyamfi and Nketsia-Tabiri (2007) for early and late morning samples, respectively. The mean bacterial counts reported in this study are lower than the values reported by Yeboah-Manu et al. (2010). In the Yeboah-Manu et al. (2010) study, mean microbial loads of 8.54 to 8.69 Log10 CFU/g was reported for salad sold with waakye on and around the University of Ghana campus, and 6.41 Log10 CFU/g from restaurants outside campus. A lot of factors may be responsible for these differences in the microbial loads reported by different authors. In the study by Yeboah-Manu et al. (2010), the salads had salad cream added to them and the salad was not heated. Salad cream contains egg yolk, which is a good medium for supporting microbial growth. In the study by Ameko et al. (2012), the salad samples used did not contain salad cream. This could account for the lower values of 4.16 Log10 CFU/g reported in their study, compared to that of Yeboah-Manu et al. (2010). According to Amoah et al. (2005 cited by Ameko et al., 2012), lettuce from vegetable farms in Accra, irrigated with drain, stream and piped water, had faecal coliform levels exceeding common guidelines for food quality, irrespective of the irrigation water source. In their study (Amoah et al., 2005), lettuce irrigated with piped water had significantly lower coliform concentrations than those irrigated with shallow well or stream water (Ameko et al., 2012).

The high bacterial contamination observed in these salad vegetables examined in this study may be reflection of storage condition and how long these vegetables were kept before they were obtained for sampling. Refrigerator storage does not necessarily inhibit the growth of microorganisms since psychrophiles, such as *Alcaligenes* and *Pseudomonas* could survive refrigeration temperatures and in some cases even multiply (Samarajeewa, 2005; Ameko et al., 2012). Bacteria on storage material may transfer to produce and cross contamination between produce is probable particular where produce are pre-washed with the same wash water by the vendor or processor. More importantly, bacteria on the vegetables may multiply over time depending on the storage condition especially those that are phsychrotrophic. According to what was reported in previous studies elsewhere inside and outside Nigeria, the initial bacteria of stored produce may have been derived from contamination of air, soil, water, insects, animals, workers and harvesting and transportation equipments (Adebayo-Tayo et al., 2012). Also, the densities of Lactic acid bacteria (LAB) in fruit and vegetable products usually range from 10^2 to 10^6 CFU/wound (Trias et al., 2008; Adebayo-Tayo et al., 2012).

The bacterial count obtained was high and these vegetables are usually consumed without heating thus there is the probability of consumers contracting pathogen if they get in contact with the vegetables. In a study by Trias et al. (2008), the highest concentrations of microorganisms were in ready-to-eat vegetables. This was due to the presence of cut surfaces, which allow higher nutrient availability (Ongeng et al., 2006) and affects not only LAB but all the microbiota related to the fresh product (Badosa et al., 2008; Trias et al., 2008; Adebayo-Tayo et al., 2012). The microbial population levels found in this study were in agreement with data reported for ready-to-eat salads in other studies (Trias et al., 2008; Adebayo-Tayo et al., 2012). A study by Amoah et al. (2007) indicated that fresh vegetables have become a normal part of fast food, served on the street, canteens and restaurants in Ghana. However, farming practices like use of contaminated irrigation water, application of manure and contaminated soils is the main source of lettuce contamination (Consultative Group on International Agricultural Research: CGIAR, 2011;

Ameko et al., 2012). An earlier study by Amoah et al. (2005) in Kumasi, Ghana indicated that 95% of lettuce samples from urban vegetable farms, irrigated with piped water, had faecal coliform levels, which were more than 1000/100 g lettuce (wet weight) and according to the International Commission on Microbiological Specifications for Food guidelines (1974), were classified as ''undesirable'' (Ameko et al., 2012).

There are several possible sources of bacterial contamination of street-vended ready-to-eat fresh salad vegetables (Ameko et al., 2012). Raw salad vegetables contamination occurs on the farm from the manure, irrigation water, contaminated hands of farmers (Centre for Food Safety, 2006), and from contaminated water used to wash the vegetables after harvest (Pavan da Silva et al., 2007; Ameko et al., 2012). The farmers may sell the vegetables at the farm, directly to the food vendors, or to market women who then sell them to the food vendors at the market (Ameko et al., 2012). If the raw vegetables are contaminated on the farm, it is highly possible that they would be consumed as such, because there is no step along the supply chain or during preparation, such as heating, for killing microorganisms (Centre for Food Safety, 2006; Ameko et al., 2012). In this study, bacterial contamination increased during the course of sale of the salad vegetables. The presence of the most frequently isolated index of food quality and indicators of faecal contamination such as *Escherichia coli*, *Enterobacter* spp. and *Salmonella* spp., is an indication of faecal contamination of the food as a result of possible unhygienic handling (Okonko *et al*., 2008a,b,c,d 2009a,b; Adebayo-Tayo et al., 2012) or contamination of the salad vegetables during processing or directly from source and this might have adverse effect on the health of the consumers (Okonko *et al*., 2008a,b,c,d, 2009a,b; Adebayo-Tayo et al., 2012).

According to Ameko et al. (2012), processing of raw vegetables into salads for sale creates conducive environments and opportunities for the multiplication of pathogenic microorganisms on the salads. In this present study, microbial contamination and counts of specific pathogens increased during sale, and the percentage of contaminated samples also increased during sale. This is because the salads still retain enough moisture to promote microbial growth, and also the natural protective covering on the leaves against the entry of microorganisms may have been lost during harvesting, storage, transport and processing (Samarajeewa, 2005; Ameko et al., 2012). The salad may also have undergone some fermentation during sale and the increased acidity may promote the growth of certain microbes such as *Bacillus cereus*, *Clostridium botullinum*, *Salmonella* sp. and *S. aureus,* which grow well in optimal pH of 4.2 to 8.2 (Samarajeewa, 2005; Ameko et al., 2012).

The isolation of *Salmonella* spp., *Escherichia coli, Klebsiella* spp. and *Serratia* spp. from these salad vegetables poses food safety problem since they are all enterotoxigenic and cause gastroenteritis. Coliforms might appear every phase of preparation; a case was reported (Seo *et al*. 2010). *E. coli* are fecal contaminants which could be from the manure in the soil on the farm (Samarajeewa, 2005; Ameko et al., 2012). The detection of *E. coli* in this study showed poor hygienic standard in the handling of these salad vegetables or it could be also be from contamination during harvest. Presence of *E. coli* indicates recent contamination by faecal matter and possible presence of other enteric pathogens known to be causative agents of food borne gastroenteritis and bacterial diarrhea disease (Adebayo-Tayo et al., 2012).

The presence of other indicator organisms like *E. coli*, *Salmonella* spp., *Shigella* spp. and *Enterobacter* spp. in salad vegetable samples might be the result of possible contamination during sales or unhygienic handling of street-vended ready-to-eat vegetables. Other studies have also identified pathogens including *Salmonella* spp. on other street foods and their accompaniment in South Africa (Mosupuye and von Holy, 1999) and Zambia (Bryan et al., 1997). While *Salmonella* spp. causes *salmonellosis* and typhoid fever, *Escherichia coli* O157:H7 causes severe illness and deaths, especially among children in several countries (WHO, 2002). *Micrococcus* sp., *Staphylococcus* spp., *Salmonella* spp., and *Shigella* spp. from contaminated hands of food handlers may easily contaminate the vegetables (Samarajeewa, 2005; Ameko et al., 2012).

The presence of *Staphylococcus auerus*, a pathogenic organism of public health concern and significance in these vegetables might have contaminated the stored vegetables from source as a result of handling by farmers or retailers (Adebayo-Tayo et al., 2012). As widely reported, most strains of *Staphylococcus aureus* are known to be pathogenic due mostly to the heat stable enterotoxin they produce in direct relationship to their inoculum level (Adebayo-Tayo et al., 2012). Considering the notoriety of the resistance of *S. aureus* to methicillin, other penicillin and celphalosporins (Adebayo-Tayo et al., 2012), its detection in salad vegetable samples poses a lot of health risk to nourishment seeking consumers. Concerning *S. aureus*, its presence suggests poor hygiene practices of operators. Bezirtzoglou *et al*. (2000) reported that the contamination by food handlers is the most common mode of transmission of this germ. Burt *et al*. (2003) established that its contamination might have resulted from man's respiratory passages, skin and superficial wounds which are his common sources.

Pseudomonas and *Bacillus* species are part of the natural flora and are among the most common vegetable spoilage bacteria. The spores of bacteria such as *Bacillus* spp., *Micrococcus* spp., etc, are carried in air and dust and if the food is not properly covered, these spores could settle on the food, and once nutrients are present, the spores can actively grow and thrive on the food (Ameko et al., 2012). The presence of *Bacillus* species in the two vegetables may be said to be due to environmental factor, the survival of Bacillus depend on several factor such as nature of the organism, resistance to the new environment and ability to form spores. Endospores of Bacillus are more resistance than their vegetable cell to harsh weather condition and even to antimicrobial treatments (Codex Alimentarius, 2007).

The contamination of salad vegetables examined in this study by pathogenic bacteria could also be as a result of poor handling practices in food supply chain, storage conditions, distribution, marketing practices and transportation (Effiuvwevwere, 2000; Okonko et al., 2008a,b,c,d, 2009a,b; Akinmusire, 2011; Akintobi et al., 2011; Adebayo-Tayo et al., 2012). Bacterial load of salad vegetables also increase with time during storage and this poses serious threat to consumers. Apart from the polythene bags, all the other containers (for example, sacks, open trays, baskets, and wooden-sieve net cages) do not protect the raw vegetables from dust and other forms of contamination from the environment during transporting from the source to the site of preparation. However, the exposure of the salad vegetables to the environment could lead to increased contamination. Improper handling and improper hygiene might lead to the bacterial contamination of salad vegetables and this might eventually affects the health of the consumers (Dunn *et al*., 1995; Omemu and Bankole, 2005; Okonko *et al*., 2008 a,b,c,d, 2009a,b; Mgbakor et al., 2011; Adebayo-Tayo et al., 2012). The condition of sales makes the vegetables predisposed to contamination especially as practiced Zaria where the source of water in the garden and in the market is questionable (Caron and Walker, 2004).

These street-vended ready-to-eat salad vegetables pose a risk to consumers because of the danger of food poisoning from microbial contamination (Food and Agriculture Organization (FAO), 2005; Ameko et al., 2012). According to Adjrah et al. (2013), the level of the microbial contamination of the ready-to-eat salads vegetables may present a potential health hazard to consumer. Certain fungi such as Aspergillus, Fusarium, and Penicillium spp. as commonly occurring filamentous fungi grow in vegetable and their growth may result in production of toxins known as mycotoxins, which can cause a variety of ill effect in human from allergic responses to immunosuppression and cancer (Adebayo-Tayo et al., 2012).

Side by side is the huge nutritional benefit derivable from consumption of these salad vegetables especially they have therapeutic, curative and preventive health uses. Although salad vegetables are commonly associated with food poisoning, they harbour disease causing organisms (Adebayo-Tayo et al., 2012); raw vegetables should not be exposed to any further contamination during transport and storage (WHO, 1996; Ameko et al., 2012). Just as with other foods, consumers have some responsibilities to carry when handling these salad vegetables. Washing of hands with warm water and soap before and after handling salad vegetables cannot be overemphasized (Adebayo-Tayo et al., 2012). Also washing of salad vegetables with salt and clean water before consumption could help in reducing microbial content eventually. However, the most efficient way to improve safety not for fruits and vegetables only, is to rely on a proactive system of reducing risk factors during production and handling. Apart from washing, other methods of decontamination seem to have a limited influence on safety (Adebayo-Tayo et al., 2012).

5. CONCLUSION

This study has further confirmed the presence of pathogens in street-vended ready-to-eat fresh salad vegetables sold in Port Harcourt, Metropolis, Rivers State, Nigeria. Vegetable can be contaminated with pathogen from animal and human reservoirs and the environment as a result of production practices. A major source of contamination is organic fertilizer (e.g. manure, municipal sludges) and faecal contaminated water. The need for microbial assessment of street-vended ready-to-eat fresh vegetables for production of food salads and for other use cannot be over emphasized to reduce possible contamination. Harvesting at the appropriate time and keeping the harvested products under well-controlled condition will help in restricting growth of pathogenic and post-harvest spoilage microorganism. Reduction of risk for human illness associated with raw product can be better achieved through controlling points of potential contamination in the field during harvesting, during processing or shipment, storage or distribution in the retail markets food services facilities or home.

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2/12/2013