Prevalence of *Salmonella* species and *Escherichia coli* in fresh Cabbage and Lettuce sold in Port Harcourt Metropolis, Nigeria

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ABSTRACT: To assess the microbial quality of cabbage and lettuce, fourteen samples of the two vegetable were purchased from different markets. In order to study the prevalence of *Salmonella spp.* and *Escherichia coli* in cabbage and lettuce, samples of these two vegetables were analyzed using standard bacteriological methods. The result revealed that out of fourteen samples examined, *Salmonella* spp. was present in 6 (42.9%) while *Escherichia coli* were present in 12 (85.7%) samples. This showed that *Escherichia coli* were most predominant over *Salmonella* spp. It showed that *Salmonella* spp. was present in samples A, B and G while *E. coli* was present in all cabbage samples. For lettuce, *Salmonella* spp. was only present in samples A, B and E while *E. coli* was present in sample A, B, C, E and G. This study has further confirmed the presence of *Escherichia coli* and *Salmonella* spp. in fresh cabbage and lettuce sold in Port Harcourt, Metropolis, Nigeria. The need for microbial assessment of these vegetables for production of food salads and for other use cannot be over emphasized to reduce possible contamination.

[Odu Ngozi Nma and Okomuda Mary Oruese. **Prevalence of *Salmonella* species and *Escherichia coli* in fresh Cabbage and Lettuce sold in Port Harcourt Metropolis, Nigeria.** *Rep Opinion*, 2013,5(3):1-8] (ISSN: 1553-9865) http://www.sciencepub.net/reports. 1

Keywords: Cabbage, Lettuce, Prevalence, *Salmonella* spp., *E. coli*

1. INTRODUCTION

Attention on vegetable as vital dietary components is significant for African population as leafy vegetable have long been known to be indispensible ingredients in traditional sauces that accompany carbohydrate staple (Gueye and Diouf, 2007). Cabbage (*Brassica oleracea capitata*) is a popular cultivar of the species *Brassica oleracea Linne* (capitata Group) of the family *Brassicaceae* (or Cruciferae) and is leafy green vegetable. It is an herbaceous, biennial, dicotyledonous flowering plant distinguished by a short stem upon which is crowded a mass of leaves, usually green (Helen, 2006). The only part of the plant is normally eaten is the leafy head; more precisely, the spherical cluster of immature leaves, excluding the partially unfolded outer leaves (Mathewson, 1998). Cabbage is used in variety of dishes of dishes for its naturally spicy flavor. It is the principal ingredients in coleslaw. In Nigeria, cabbage is used in varieties of dishes such as salad, coleslaw, fried rice, jollof rice, shawamah, in place of onion in suya and in some pastries. This parcel of vegetable is nutrient-packed and low in calorie. It is impressive with its high content levels of calcium, iron, potassium, sulfur, and phosphorus. Cabbages are also a good source of riboflavin (Mathewson, 1998). Cabbage is a good source of beta-carotene, vitamin C and fiber. It is a cruciferous vegetable, and has been shown to reduce the risk of some cancers, especially those in the colorectal group. This is possibly due to the glucosinolates found in cole crops, which serve as metabolic detoxicants, or due to the sulphoraphane content, also responsible for metabolic anti-carcinogenic activities. Purple cabbage also contains anthocyanins, which in other vegetables have been proven to have anti-carcinogenic properties (Katz and Weaver, 2003). Along with other cole crops, cabbage is a source of indole-3-carbinol, a chemical that boosts DNA repair in cells and appears to block the growth of cancer cells (Fan et al., 2006; Wu et al., 2010). Research suggests that boiling these vegetables reduces their anti-carcinogenic properties (Warwick Medical School, 2007).

In addition to its usual purpose as an edible vegetable, cabbage has been used historically as a medicinal herb. The ancient Roman nobleman Pliny the Elder described both culinary and medicinal properties of the vegetable, recommending it for drunkenness–both preventatively to prevent the effects of alcohol, and to cure hangovers (Dalby and Grainger, 1996). This traditional usage persisted in European literature until the mid-20th century (Hatfield, 2004). In Cato the Elder's work *De Agri Cultura* ("On Agriculture"), he suggested that women could prevent diseases in their private parts by bathing in urine obtained from those who had frequently eaten cabbage (Wright, 2001). The cooling properties of the leaves were used in Britain as compresses for ulcers and breast abscesses, and as a

treatment for trench foot in World War I. Other medicinal uses recorded in Europe folk medicine include treatments for rheumatism, sore throat, hoarseness, colic, and melancholy (Hatfield, 2004).

In the United States, cabbage has been used as a hangover cure, to treat abscesses, to prevent sunstroke, or to cool body parts affected by fevers. The leaves have also been used to sooth sore feet, and, tied around the neck of children, to relieve croup. Mashed cabbage and cabbage juice have been used in poultices to remove boils and treat warts, pneumonia, appendicitis, and ulcers (Hatfield, 2004).

However, cabbage has been linked to outbreaks of some food-borne illnesses, including *Listeria monocytogenes* (Davis and Kendall, 2012) and *Clostridium botulinum*. The latter toxin has been traced to pre-made, packaged coleslaw mixes, while the spores were found on whole cabbages that were otherwise acceptable in appearance. *Shigella* species are able to survive in shredded cabbage (US Food and Drug Administration, 2012). Two outbreaks of *E. coli* in the United States have been linked to cabbage consumption. Biological risk assessments have concluded that there is the potential for further outbreaks linked to cabbage, due to contamination at many stages of the growing, harvesting and packaging processes. Contaminants from water, humans, animals and soil have the potential to be transferred to cabbage and from there to the end consumer (Ontario Ministry of Agriculture, Food and Rural Affairs, 2001).

Lettuce (*Lactuca sativa*) is a temperate annual or biennial plant of the daisy family Asteraceae. It is most often grown as a leaf vegetable. It is eaten either raw, notably in salads, sandwiches, hamburgers etc. Lettuce is a common vegetable most known for its widespread use in salads and on sandwiches vegetables, like lettuce are healthy to eat because they are low in calories and contain essential nutrients (Gueye and Diouf, 2007) Many of the phytonutrients found in green, leafy vegetable are antioxidants, which fight cancer and heart disease (Haber, 2004).

Depending on the variety, lettuce is a good source of vitamin A, vitamin K and potassium, with higher concentrations of vitamin A found in darker green lettuces. It also provides some dietary fiber (concentrated in the spine and ribs), carbohydrates, protein and a small amount of fat. With the exception of the iceberg type, lettuce also provides some vitamin C, calcium, iron and copper, with vitamins and minerals largely found in the leaf (Katz and Weaver, 2003).

In addition to its usual purpose as an edible leafy vegetable, lettuce has had a number of uses in ancient (and even some more modern) times as a medicinal herb and religious symbol. For example, ancient Egyptians thought lettuce to be a symbol of sexual prowess (Katz and Weaver, 2003) and a promoter of love and childbearing in women. The Romans likewise claimed that it increased sexual potency (Watts, 2007). In contrast, the ancient Greeks connected the plant with male impotency (Weaver, 1997), and served it during funerals (probably due to its role in the myth of Adonis's death), and British women in the 1800s believed it would cause infertility and sterility. Lettuce has mild narcotic properties – it was called "sleepwort" by the Anglo-Saxons because of this attribute – although the cultivated *L. sativa* has lower levels of the narcotic than its wild cousins (Watts, 2007). This narcotic effect is a property of two sesquiterpene lactones which are found in the white liquid (latex) in the stems of lettuce called lactucarium or "lettuce opium" (Katz and Weaver, 2003).

Lettuce extracts are sometimes used in skin creams and lotions for treating sunburn and rough skin. It was once thought to be useful in relieving liver issues. Some American settlers claimed that smallpox could be prevented through the ingestion of lettuce (Watts, 2007), and an Iranian belief suggested consumption of the seeds when afflicted with typhoid (Duke et al., 2007). Folk medicine has also claimed it as a treatment for pain, rheumatism, tension and nervousness, coughs and insanity; scientific evidence of these benefits in humans has not been found, although some similar effects have been demonstrated in mice and toads (Katz and Weaver, 2003). The religious ties of lettuce continue into the present day among the Yazidi people of northern Iraq, who have a religious prohibition against eating the plant (MacFarquhar, 2003).

Although most food-borne pathogens can survive on stored lettuce, they tend to decline in number during the storage period. The exception to this is *Listeria monocytogenes*, the causative agent of listeriosis, which multiplies in storage. However, despite very high levels of the bacteria being found on ready-to-eat lettuce products, a 2008 study found no incidences of food-borne illness related to listeriosis. The researcher posited that this may be due to the product's short shelf life, indigenous microflora competing with the *Listeria* bacteria or possible properties within the lettuce that cause the bacteria to be unable to cause listeriosis (Hanning et al., 2008).

Other bacteria found on lettuce include *Aeromonas* species, which have not been linked to any outbreaks; *Campylobacter* species, which cause campylobacteriosis and *Yersinia intermedia* and *Yersinia kristensenii* (species of *Yersinia*), which have been found mainly in lettuce (US Food and

Drug Administration, 2012). Lettuce has been linked to numerous outbreaks of the bacteria *E. coli O157:H7* and *Shigella*; the plants were most likely contaminated through contact with animal feces (Davis and Kendall, 2012). A 2007 study determined that the vacuum cooling method, especially prevalent in the California lettuce industry, increased the uptake and survival rates of *E. coli O157:H7* (Li et al., 2008). *Salmonella* bacteria, including the uncommon *Salmonella braenderup* type, have also caused outbreaks traced to contaminated lettuce (Gajraj et al., 2012). Viruses, including hepatitis A, calicivirus and a Norwalk-like strain, have been found in lettuce. The vegetable has also been linked to outbreaks of parasitic infestations, including *Giardia lamblia* (US Food and Drug Administration, 2012).

Microorganisms are naturally present on all foodstuffs and can also be brought in by outside elements (wind, soil, water, insects, animals, human handling. They can become contaminated during growing, harvesting and transport of the raw materials, and/or processing into finished products (Lelieveld *et al.*, 2003). Cabbage and lettuce comprise a diverse range of plant parts (leaves, roots, tubers, fruits, and flowers). Production practices, growth condition and the location of the edible part during growth (soil , soil surface, and aerial part) will in combination with intrinsic, harvesting and processing factors affect their microbial status at the time of consumption. The quality of the water used for irrigation may also serve as a source of contamination (Ibenyassine, 2007). Technologies for irrigation are important for the control of spreading microbiological hazards. However, heavy rains and wind may provide other opportunities for the transfer of microorganism from soil to plant surfaces. The increase in demand on the market for organically grown fruits and vegetables has resulted in the use of organic fertilizers and the use of alternative measure to chemical plant protection products for the control of pests, mites and fungi. Therefore, the potential risks for contamination of organically grown fruits and vegetable by faecal pathogen or by mycotoxin producing moulds may be a source of contamination (Nguyen, 1994).

The genus S*almonella* has over 2700 serotypes. Animals and birds are the natural reservoirs. *Salmonella* is a rod-shaped, motile bacterium-nonmotile exceptions *S. gallinarum and S. pullorum* –non spore forming and Gram-negative bacteria. *Salmonella* is associated mainly with raw meats, poultry and dairy products. However, many other foods have been implicated in outbreaks caused by *Salmonella*, and these include mayonnaise, salads, milk, orange juice, sprouted seeds and dairy products etc (Beuchat, 1995). The source of *Salmonella* contamination may include soil, raw or improperly composted manure, irrigation or wash water, handling by workers and contact with equipment surface. The persistence and survival of *Salmonella* on produce is affected by its ability to adapt to the new ecological environments (Beuchat, 2002).

Escherichia coli O157:H7 is major causes of food borne disease in the Africa. Leaf vegetables (lettuce and cabbage), have been implicated in several large outbreaks of food born disease caused by *E. coli* O157:H7, a pathogen with increasing public health significance because of the severity of the gastrointestinal illness and the long-term, chronic sequelae that can result from infection. Contamination of raw produce with these pathogenic microorganisms can occur at many points in the food continuum, from the field to the market (Barak *et al.*, 2002). Diarrhoea and haemolytic uraemic syndrome (HUS) are caused by *E. coli* strains. *Escherichia coli* commonly abbreviated (*E. coli*) is a Gram-negative, rod- shaped bacterium that is commonly found in lower intestine of warm –blooded organism (endotherms). Most *E. coil* strains are harmless, but some serotype can cause serious food poisoning in human. The harmless strains are part of the normal floral of the gut, and can benefit their hosts by producing vitamin k_2 and by preventing the establishment of pathogenic bacteria within the intestine (Howard, 2002).

The present study was undertaken in order to determine the prevalence of *Escherichia coli* and *Salmonella* spp. in fresh cabbage and lettuce sold 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 cabbage and lettuce 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. Isolation and Identification of 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 was pipetted out and pour plated using MacConkey agar (MCA) and Salmonella-Shigella agar (SSA). These plates were incubated at 37^{0}C 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 vegetables (cabbage and lettuce) are shown in Tables 1 to 2.

3.1. Frequency of occurrence of *Salmonella* spp. and *E. coli* Isolates

Table 1 shows the frequency of occurrence of *Salmonella* spp. and *E. coli* Isolates associated with vegetables in Port Harcourt metropolis, Nigeria. It showed that *Escherichia coli* (77.1%) were most predominant over *Salmonella* spp. (22.9%).

Table 1: Frequency of occurrence of *Salmonella* spp. and *E. coli* Isolates associated with cabbage and lettuce in Port Harcourt Metropolis, Nigeria

Isolates	No. (%)	Cabbage (%)	Lettuce (%)
Escherichia coli	54(77.1)	43(79.6)	11(20.4)
Salmonella spp.	16(22.9)	8(50.0)	8(50.0)
Total	**70(100.0)**	**51(72.9)**	**19(27.1)**

3.2. Prevalence of *Salmonella* spp. and *Escherichia coli* in cabbage and lettuce samples

The prevalence of *Salmonella* spp. and *Escherichia coli* in cabbage and lettuce samples is shown in Table 2. The result revealed that out of fourteen samples examined, *Salmonella* spp. was present in 6(42.9%) samples while *Escherichia coli* were present in 12(85.7%) samples. It showed that *Salmonella* spp. was present in samples A, B and G while *E. coli* was present in all cabbage samples. More so, for lettuce, *Salmonella* spp. was only present in samples A, B and E while *E. coli* was present in sample A, B, C, E and G (Table 2).

Table 2: Prevalence of *Salmonella* spp. and *Escherichia coli* in cabbage and lettuce samples

Samples	Type	*Escherichia coli* (%)	*Salmonella* spp. (%)
A	Cabbage	8(14.8)	3(18.8)
B	Cabbage	10(18.5)	1(6.3)
C	Cabbage	5(9.3)	0(0.0)
D	Cabbage	3(5.6)	0(0.0)
E	Cabbage	3(5.6)	0(0.0)
F	Cabbage	2(3.7)	0(0.0)
G	Cabbage	3(5.6)	4(25.0)
A	Lettuce	7(12.9)	3(18.8)
B	Lettuce	7(12.9)	1(6.3)
C	Lettuce	3(5.6)	0(0.0)
D	Lettuce	0(0.0)	0(0.0)
E	Lettuce	2(3.7)	4(25.0)
F	Lettuce	0(0.0)	0(0.0)
G	Lettuce	1(1.9)	0(0.0)
Total		**54(100.0)**	**16(100.0)**

4. DISCUSSION

The present study aimed at assessing the prevalence of *Salmonella* spp. and *Escherichia coli* in commonly consumed cabbage and lettuce vegetables sold in Port Harcourt metropolis, Nigeria. The result showed that out of fourteen samples examined, *Salmonella* spp. was present in 6(42.9%) samples while *Escherichia coli* were present in 12(85.7%) samples. The prevalence of *Salmonella* spp. was also 50.0% for cabbage and 50.0% for lettuce, while *Escherichia coli* were 79.6% and 20.4% for cabbage and lettuce respectively. This result showed that *E. coli* was most predominant over *Salmonella* spp. and other microorganisms isolated from vegetable samples examined. However, these pathogens in the vegetables may have been a direct reflection of sanitary quality of the cultivation water, harvesting, transportation, storage, and processing of the plant produce (Beuchat, 1996). According to a study by Amponsah-Doku et al. (2010) on the bacterial contamination of lettuce and at production sites, markets and street-food restaurants in the city of Kumasi, Ghana in general, the levels of thermo-tolerant coliforms on lettuce was increased by 18.0%, while *Enterococci* numbers reduced by 64.0% from the farms to the street-foods.

In this study, *Salmonella* spp. was present in samples A, B and G while *E. coli* was present in all cabbage samples. More so, for lettuce, *Salmonella* spp. was only present in samples A, B and E while *E. coli* was present in sample A, B, C, E and G. This contrary to the findings of Adjrah et al. (2013) who reported that *Salmonella* spp. was not detected in any of the samples evaluated in their study. Our present finding is also contrary to what was reported in our previous study on shawarma in that only *E. coli* was isolated and none of the samples from the three locations had any growth of *Salmonella* spp. 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 isolation of *Salmonella* spp. and *Escherichia coli* from cabbage and lettuce poses food safety problem since they are all enterotoxigenic and cause gastroenteritis. 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 (Jiwa et al., 1981; Adebayo-Tayo et al., 2012). Other studies have also identified pathogens including *Salmonella* spp. on other street foods and their accocmpaniment 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).

There are several possible sources of contamination of street-vended ready-to-eat vegetables (Ameko et al., 2012). 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). *Salmonella* spp. from contaminated hands of food handlers may easily contaminate the vegetables (Samarajeewa, 2005; Ameko et al., 2012). The presence of the most frequently isolated index of food quality and indicators of faecal contamination such as *Escherichia coli* and *Salmonella* spp., reported in this study is an indication of faecal contamination of the food as a result of possible unhygienic handling (Okonko *et al*., 2008 a,b,c,d 2009a,b; Adebayo-Tayo et al., 2012) or contamination of the vegetable itself 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).

Although vegetables are commonly associated with food poisoning, they harbour disease causing organisms (Adebayo-Tayo 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 (Ameko et al., 2012). 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 *Salmonella* spp. which grow well in optimal pH of 4.2 to 8.2 (Samarajeewa, 2005; Ameko et al., 2012).

5. CONCLUSION

This study has further confirmed the presence of *Escherichia coli* and *Salmonella* spp. in fresh cabbage and lettuce vegetables sold in Port Harcourt, Metropolis, 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 these vegetables for production of food salads and for other use cannot be over emphasized to reduce possible contamination.

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2/12/2013