

Bacterial Contamination of *Lactuca sativa*, *Spinacia olerencea* and *Brassica olerencea* in Kano Metropolis

D.W. Taura, T.I. Oyeyi and L.D. Fagwalawa

Department of Biological Sciences, Bayero University, P.M.B. 3011, Kano, Nigeria

Abstract: The study was conducted to determine various bacterial species responsible for the contamination of leafy vegetables in Kano metropolis. Three leafy vegetable samples namely, *Brassica olerencea* (cabbage), *Lactuca sativa* (Lettuce) and *Spinacia olerencea* (Spinach) were obtained from different markets. Bacterial species isolated were *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus* and *Streptococcus* sp. The results have shown that out of the samples examined *Staphylococcus* sp. accounts for a high percentage of occurrences with 42.5% followed by *Escherichia coli* having 27.9%, then *Pseudomonas aeruginosa* with 22 and 7.5% for *Streptococcus* which has been the least. Therefore, consumption of these types of vegetables unhygienically paves way for ingestion of considerable numbers of human pathogenic bacteria. This ultimately results in establishment and manifestation of diseases in the final host.

Key words: Bacterial contamination, *lactuca sativa*, *spinacea olerencea*, *brassica olerencea*

INTRODUCTION

Vegetables are any kind of plant product. They refer to the fresh edible portion of herbaceous plant roots, stems, leaves or fruits (Encyclopedia, 1968). Vegetables are mostly annual crops belonging to the group of plants called horticultural crops which are diverse in nature. Vegetables can be grouped into fruit and leafy vegetables depending on the nature of their consumable products or parts.

Fruit vegetables are those that produce fruits such as Okro, Tomatoes, Garden egg e.t.c. while leafy vegetables are those whose leaves are the desired parts e.g. lettuce, spinach, cabbage, cauliflower, parsley, e.t.c, on which this research is based.

Both types are usually harvested green for human consumption when fresh they have a high water content of about 80% (NSPRI, 1992). Living creatures more especially humans depend on vegetables as a source of food for their living. But some of these vegetables inflict economic loss by causing or spreading of human disease after consumption and these affect the health and the progress of the nation. Vegetables possess a high content of minerals and vitamins. According to Matthew (1985) green vegetables are considered as good source of vitamins, minerals such as copper and iron. Their soft textured nature makes them highly attractive to microbial invasion and they are very susceptible to physical and microbial spoilage. The common sources of

microorganisms that contaminate leafy vegetables include air, soil, farm pests (nematodes), handlers, irrigation containers used e.t.c. (Center for overseas pest research, 1986). Leafy vegetables can become contaminated whilst growing in the field or during harvest, handling, processing, distribution and use (Beuchat, 1998). Any microbial contamination present is likely to reflect the environment through which the product has passed.

Microbiologically, irrigated vegetables are found to be highly contaminated with bacteria that are harmful to both plants and animals including man (Matthew, 1985). Consumption of these types of vegetables unhygienically paves way for ingestion of considerable number of human pathogenic bacteria. This eventually results in establishment and manifestation of disease on the final host. The presence of cut and damage can provide an opportunity for contamination and growth of microorganisms into plant tissues (Francis and O' Beirne, 1999). In view of the fact that there are many different types of illnesses associated with eating leafy vegetables, it is the aim of this study to investigate the bacterial contaminants to these leafy vegetables.

MATERIALS AND METHODS

Sample collection: Different leafy vegetable samples were collected in a sterile polythene bag to make aseptical collection and a sterile hand glove was used. The samples were collected for a period of 3 weeks. They include the

following Lettuce (*Lettuce sativa*), Spinach (*Spinaca olerencea*) and Cabbage (*Brassica oleracea*) from Sabongari, Rimi and Yankaba markets. Each sample was carried to the laboratory immediately following collection for bacteriological examination.

Bacterial examination: The leaves of vegetables were selected and washed with distilled water. The washed leaves were also grounded and then diluted with peptone water. Bacteriological analysis of the vegetables was carried out immediately in accordance with the methods of American Public Health Association (APHA, 1988) using modified pour plate technique.

The samples were mixed with the nutrient agar before it sets so the colonies will spread throughout the medium instead of growing only on the surface. Serial dilution of the samples of 10^{-1} through to 10^{-5} was made.

Five cotton plugged sterile test tubes were appropriately labeled and serially arranged on the test tube rack for each sample. Nine millilitre of peptone water was introduced into each test tube using sterile (10 mL) pipette. Using separate sterile pipette i.e., about 1 mL of the grounded test sample was introduced into the first test tube (10^{-1}). It was mixed thoroughly using a mixed 1 mL of the first test tube was aseptically pipetted and introduced into the second test tube (10^{-2}). The same procedure was done for the rest of the test tubes. As soon as the serial dilution was completed, 1ml of each of the dilution was aseptically pipetted and introduced into corresponding labeled sterile Petri dishes. The agar was poured slowly into the base of the sterile Petri dish with slight swirling for uniform distribution of the medium.

On completion of the inoculation, the plates were allowed to solidify after which they were incubated at a temperature of 37°C . The plates were read and recorded after 24 h and the results expressed in colony forming unit per millimeter of the test sample.

IDENTIFICATION TECHNIQUES USED

Gram staining: Gram staining is important to differentiate between gram positive and gram negative bacteria. Before staining all bacteria are colourless, afterwards gram positive bacteria stained violet and gram negative bacteria red. The difference between the two types of bacteria were due to the differences in their cell wall structures. These organisms which after being stained dark purple with crystal violet (basic stain) are not decolorized by acetone or ethanol are called gram positive while those after being stained with crystal violet lose their colour when treated with ethanol and stain red with safarin (counter stain) are called gram negative.

Catalase test: It is used to detect the presence of catalase in a given stain of bacterium. Catalase is an iron containing enzyme which catalyses the decomposition of H_2O_2 and O_2 . It is formed by most aerobic bacteria. This test helps to differentiate *Staphylococcus* sp., from *Streptococcus* sp. using hydrogen peroxide (H_2O_2). After using a sterilized wire loop to take a streak of good colony of the test organisms unto a slide containing drop of the reagent. Air bubbles in other words, evolution of gas indicates positive result.

Indole test: This test detects ability of organisms to produce indole from amino acid tryptophan. It is also important in the differentiation of coli forms and depends on the production of indole on tryptophan. Following an incubation of the test organisms in peptone water, agar for 24 h. Few drops of Kovac's reagent were added, shaken and allowed to stand. The appearances of red colouring which separated out with the alcohol layer indicates the positive result.

Oxidase test: This test was used to differentiate *Pseudomonas* sp. from other gram negative enteric bacteria is based on the presence of a few drops of the enzyme indo-phenol Oxidase was added onto the colonies of the test organisms, Oxidase positive colonies quickly become dark-purple within 10 sec.

RESULTS

The results of the bacteriological examination of the different vegetable samples from various markets in Kano metropolis (Sabongari, Rimi and Yankaba) are presented in Table 1-5.

Table 1 shows the number of bacterial colonies per ml of the sample in mean colony expressed in terms of colony

Table 1: Bacterial mean colony count (CFU mL^{-1}) in each vegetable

Sample	Nutrient ($\times 10^7$)	Mac conkey agar ($\times 10^7$)
Lettuce	1.17	1.3
Cabbage	6.95	2.5
Spinach	2.05	2.02

Table 2: The organisms isolated and their laboratory features

Organisms isolated	Pigment	Gram stain	oxidase	catalase	indole
<i>Pseudomonas seruginosa</i>	Pyocyanin (green pigment)	Gram negative bacilli	negative	+	-
<i>Staphylococcus</i>		Gram positive cocci	-	+	-
<i>Streptococcus</i>		Gram positive cocci	-	-	-
<i>Escherichia coli</i>		Gram negative rod	-	-	+

Table 3: Percentage of occurrence (%) of bacterial species in each vegetable examined

Bacterial contaminant	Cabbage	Lettuce	Spinach
<i>Pseudomonas</i>	42	33	40
<i>Staphylococcus</i>	66	86	70
<i>Streptococcus</i>	16	13	10
<i>Escherichia coli</i>	43	50	53

Table 4: Frequency of occurrence (%) of bacterial species in each vegetable examined

Bacterial contaminant	Cabbage	Lettuce	Spinach
<i>Pseudomonas</i>	10	12	8
<i>Staphylococcus</i>	25	28	26
<i>Streptococcus</i>	4	5	00
<i>Escherichia coli</i>	12	20	15

forming unit. Cabbage has the highest mean of 6.95×10^7 on nutrient agar and lettuce having the lowest of 1.17×10^7 on mac Conkey agar. The results of the identification tests such as gram staining, indole, catalase and oxidase techniques used to differentiate the organism isolated from the samples were presented in Table 2. Table 3 shows the results of percentage of occurrence by bacterial species in each vegetable examined. The highest and lowest percentage contamination was found to be 86% by *Staphylococcus* and 10% by *Streptococcus*, respectively. The results of frequency of occurrence by bacterial species in each vegetable are shown in Table 4. The most frequent bacteria isolated from the examined vegetable were *Staphylococcus* and *Escherichia coli* having the highest frequency in all the samples examined. And the least frequent bacterial specie was *Streptococcus* having the lowest frequency of contamination in cabbage. Table 5 show the results of the total percentage of occurrence in the overall vegetables examined. *Staphylococcus* sp. was found to have the highest percentage in the total sampled with 74% and *Streptococcus* sp. having the lowest frequency of 13%, respectively.

DISCUSSION

The results have shown that contamination is by *Staphylococcus*, *Streptococcus* and *Pseudomonas* and *Escherichia coli*. In all the samples examined, cabbage was found to be the most contaminated followed by lettuce and spinach in decreasing order (Table 1).

Among the isolates, *Staphylococcus* sp was found to be most prevalent with average percentage of occurrence of 54% (Table 5). This does not comply with Gunol's (1996) findings where *Staphylococcus* sp. obtained 14% contamination rate. The high frequency of *Staphylococcus* sp. in this investigation is possibly because they are present as normal flora of humans and contaminate the vegetables as a result of poor hygiene and unsatisfactorily sanitation.

Table 5: Percentage of occurrence (%) by individual bacterial species

Bacterial contaminant	Total percentage contamination (%)
<i>Pseudomonas</i>	22
<i>Staphylococcus</i>	42.5
<i>Streptococcus</i>	7.5
<i>Escherichia coli</i>	27.9

Occurrence of *E. coli* on fresh produce may also be as a result of field contamination because of water run off from wild animals (Hill born *et al.*, 1999; Rice *et al.*, 1999). And high account of *Escherichia coli* was reported on vegetables grown where untreated water is the main source of water for the vegetables. 50% of the vegetables were found to be contaminated with *Escherichia coli* (McDonald, 1996). This shows almost similar relation with the results obtained in this research where *E. coli* accounts for 48.6% of occurrence (Table 5).

Some pathogenic organisms can have environmental sources although these are rarely defined for example *Pseudomonas* may come from the environment, water, or raw vegetables (Kominos *et al.*, 1997). *Pseudomonas* sp. were also isolated and identified due to its pigment i.e. pyocyanin (green pigment). In this investigation 30% of the samples examined were found to be contaminated with *Pseudomonas aeruginosa*. The reason linked to this contamination is that the main vegetables farm center situated at Kwakwachi makes use of domestically and industrially polluted *Streptococcus* sp. was found in low percentage (13%) of the samples examined.

The results indicated a relationship between the microbiological quality of the water used for these vegetables and the extent of human and other animal defecation, agricultural activities as well as domestic and industrial discharge being emptied continuously into these bodies of water. Thus, the occurrence of large numbers of coli form bacteria in this study might be as a result of possible opportunistic occurrence or proliferation which could have also allowed the multiplication of *Escherichia coli*. The establishment of the presence in sufficient numbers of organisms which are not necessary pathogens but are obligate inhabitants of the human intestine only serves as an evidence of fecal contamination of these water supplies used for irrigating the farms. These supplies can therefore be regarded as potential carriers of enteric pathogens.

The vegetables were exposed in the market without proper sanitary care, refuse or waste were dumped everywhere and even the water used for sprinkling the vegetables is industrially or domestically polluted all this may be the source of contamination of the vegetables.

RECOMMENDATIONS

Special concern has to be taken from application of fresh manure as fertilizer. The potential of organic farming

to contaminable vegetables pathogens has to be investigated. Water used in the production, irrigation and washing vegetables should be at a level that does not introduce microorganisms that might cause harm to the consumers. Vegetables most especially leafy vegetables that are eaten raw should be properly washed with salt or vinegar before eating.

CONCLUSION

In conclusion, vegetables can be contaminated with pathogens from animal and human reservoirs and the environment as a result of production practices. Harvesting at the appropriate time and keeping the harvested products under well controlled conditions will help in restricting growth of pathogens and post harvest spoilage microorganisms. The use of additional post harvest procedures can reduce the contamination level on leafy vegetables. The most efficient way to improve safety to leafy vegetables is to rely on a proactive system reducing risk factors during production and handling.

REFERENCES

- American Public Health Association APHA, 1988. Recommended methods for the examination of food products. (13th Edn.), Washington. DC.
- Beuchat, L.R., 1998. Pathogenic microorganism associated with fresh Produce. J. Food Protec. Center for overseas pest research 1989.
- Encyclopedia Britannica, 1968. Vegetables and cultivation, 22: 173-178.
- Francis, G.A., Thomas and T. Obeirne, 1999. The microbiological safety of minimally processed vegetables. Int. J. Food Sci. Tech., 34: 1-22.
- Gonul, M.C., 1996. Microbiological quality control of food product. Turkish J. Biol., 20: 263-268.
- Hill Born, E.D., J.H. Merman, P.A. Msher, J.Z. Hedler and A. Voestch, *et al.*, 1999. A multistate outbreak of *Escherichia coli* 0157:117 infections associated with consumption of mesclun lettuce. Arch. Int. Med., 159: 1758-1764.
- Kimono's, S.D., C.E. Copeland, B. Grosiak and B. Postic, 1997. Introduction of *Pseudomonas aeruginosa* into a hospital in a vegetable. Applied Microbiol., 24: 567-70.
- Mathew, C.S., 1985. Microbiological quality of food product. J. Food Protec., 654: 304-309.
- McDonald, G. H., 1996. Epidemiology of infection due to *Escherichia coli*. A 3 year prospective study of epidemiology and infection, 116: 279-284.
- N.A.C.M.F., 1999a. National Advisory Committee on Microbiological criteria for foods, Microbiological safety education and recommendation on fresh produce.
- Nigeria Stored Products Research Institute, 1992. Storing your produce. Advisory booklet.
- Rice, D.H., D.P. Hancock and Besse, 1999. Verotoxigenic *E. coli* 0157 colonization of wild Deep and range. Cattle Vet. Res., 137: 524-34.