Microbial Qualities of Vegetables, Water and Soils From Vegetable Gardens In Lagos State, Nigeria

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Abstract: The presence of coliforms in vegetables, water and soil samples from gardens in Lagos State was assayed for. The vegetables sampled were cabbage, waterleaf, carrot, lettuce and cucumber collected from five sites representing five geograpical zones. The isolates obtained were cultured on MacConkey (MAC) agar, Sorbitol MacConkey agar, Eosine Methylene Blue (EMB) agar and *Salmonella-Shigella* (SSA) agar. They were identified using morphological, biochemical and Analytical Profile Index 20E and 20SA kit. Hemolytic activity of the isolates was assayed for using Blood agar. The aerobic plate counts of soil, water and vegetable samples ranges from 8.80 x 10^7 to 8.00 x 10^9 cfu/g, 3.90 x 10^9 to 6.15 x 10^9 cfu/ml, and 3.30 x 10^9 to 1.08 x 10^{10} cfu/g, respectively. Coliforms were the predominant bacteria isolated from the sites. The coliform counts of cabbage, waterleaf, carrot, lettuce and cucumber were5.01 x 10^9cfu/g, 6.76 x 10^7cfu/g, 5.49 x 10^7cfu/g, 1.58 x 10^8cfu/g and 4.67 x 10^4cfu/g respectively. The fecal colifom population range was between 2.51 x 10^3 and 1.31 x 10^8 cfu/g, while *Salmonella* and *Shigella* species ranged from 1.38 x 10^2 - 3.09 x 10^4 cfu/g. *Escherichia coli* 0157:H7 was not isolated from any of the sites. The study showed that washed vegetableswere contaminated with high microbial load especially coliforms.

Keywords: Coliforms, hemolysis, soil, vegetables, water

Introduction

Coliform bacteria are commonindicators of sanitary quality of foods and water. They can be found in aquatic environment, in the soil and on vegetation. They have been implicated in some cases of life-threatening infections and their presence is used to indicate that other pathogenic organisms of fecal origin may be present (Kim and Harrison, 2008).

Food borne illness has recently gained much attention worldwide due to its deleterious effects on human health and consequentially on national economy. Worldwide increased consumption of fresh vegetables in the form of raw and minimally processed salads has resulted in increase in food borne outbreaks which sometimes may be fatal (Little and Gillespie, 2008). Food borne outbreaks are witnessed by people of developed countries like the United States, Japan and Germany (Johnston *et al.,* 2006). Outbreaks of food-borne illnesses associated with consumption of contaminated vegetables have been linked to *Escherichia coli*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Shigella*spp. and *Salmonella* spp. (Johnston *et al.,* 2006; NSW, 2007). Coliform bacteria, particularly *E. coli*, are an index of water and food sanitation. Some of the strains are pathogenic like *E. coli* O157:H7 and are able to produce serious illness in humans including diarrhea, vomiting, severe abdominal pain, hemorrhagic colitis (HC), and the acute hemolytic uremic syndrome (HUS). Vegetables can become contaminated with pathogenic organisms during growth, harvesting, post-harvest handling, or distribution (McMahon and Wilson, 2001).

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Some food borne outbreaks have been reported to be due to field contamination before these greens are harvested (SGM, 2007). The use of untreated wastewater in irrigation represents an important route for transmission of these pathogenic organisms.

The aim of this study was therefore to determine the microbial quality of vegetables, water and soils from vegetable gardens in Lagos State, Nigeria and to evaluate the prevalence of coliforms in the vegetables.

Materials and Methods

Sources of Samples

This study was carried out on different vegetable gardens and at six different locations (Oko-Oba, IyanoIba, Ikorodu, Mushin, Idi-Araba and Epe) with different proximities to refuse dump sites and to areas with human population in Lagos state, Nigeria. Samples of vegetables, soils and water were collected once from each location and were transported in ice bags to the laboratory for immediate analysis or stored at 4ºC prior to analysis.

Sample Preparation

The vegetable samples were soaked for one minute in 70% alcohol so as to eliminatesurface microorganisms, rinsed with sterile distilled water and then shredded to pieces. The vegetableswere aseptically homogenized in distilled water using sterile pestle and mortar. At each location, the soil samples were collected from five different points in the garden and the collected samples composited. Samples of the following water sources used in watering the gardens in their respective locations were collected aseptically for analysis: Idi-Araba (irrigation pond), Oko-oba (rain

water), Iyana-Iba (well water), Epe (treated tap water), Ikorodu (irrigation pond), and Mushin (well water).

Isolation And Identification Of Microorganisms

Aliquots of 0.1 ml from a ten-fold serial dilutions were inoculated in triplicates into freshly prepared growth media using the spread plate technique. Nutrient agar (NA), MacConkey (MAC) agar, Eosine Methylene Blue (EMB) agar and *Salmonella-Shigella* (SSA) agar were incubated at 37^{o}C for 24 hours. Potato Dextrose agar (PDA) was incubated at 28^{o}C for 48-72 hours. After incubation, the colonies that developed were enumerated. The bacterial isolates that grew on EMB Agar and SSA Agar were identified using morphological, biochemical and Analytical Profile Index 20E and 20SA kit.

Isolation of Escherichia Coli 0157:H7

Escherichia coli 0157:H7 was isolated using Sorbitol MacConkey agar which supports its growth. All the isolates that grew on EMB agar were streaked on Sorbitol MacConkey agar andincubated at 37^{o}C for 24 hours. After the incubation, the plates were observed for growth of colonies and colour changes. Pink coloration on the plate indicates the growth of other stains of *Escherichia coli*,while colourless growth indicates the growth of *Escherichia coli* 0157:H7.

Hemolysis Test

This test is dependent on the presence of a substance called hemolysin, which breaks down red blood cells. The bacterial isolateswere streaked on Blood agar, incubated at 37^{o}C for 24-28 hours and observed for hemolysis of red blood cells. A positive result (β-hemolysis)is indicated by the complete lyses of red blood cells in the media around and under the colonies (the area appears lightened and transparent). When the agar under and around the colony is unchanged (non-hemolytic), the result is negative (γ-hemolysis). A partial hemolysis (α-hemolysis) is present when the agar under the colony is dark and greenish.

Results

The distribution of the microbial populations in water, soil and vegetables in gardens across different locations in Lagos State is presented in Figure 1. The total heterotrophic bacterial populations ranged between 3.90 x 10^{9} – 6.15 x 10^{9}cfu/ml and 8.80 x 10^{7} - 1.08 x 10^{10}cfu/g for water and soil and vegetables respectively. The growths on McConkeyagar and Eosine Methylene Blue agar were between 5.03 x 10^{3} – 1.33 x 10^{8}cfu/g and 3.60 x 10^{5} – 1.24 x 10^{7}cfu/g respectively. The populations of the total heterotrophic fungi and *Salmonella* and *Shigella* species ranged between 6.00 x 10^{3} – 7.70 x 10^{7}cfu/g and 1.41 x 10^{1} – 8.14 x 10^{7}cfu/g respectively. Statistically, there were no significant differences in the bacterial populations among the samples for these sites. Also, the population of total coliforms of vegetables presented in Figure 2 ranged between 4.67 x 10^{4} – 5.01 x 10^{9}cfu/g while the growth on EMB agar was between 2.51 x 10^{3} – 1.31 x 10^{8}cfu/g. The population of *Salmonella* and *Shigella* species was however low (1.38 x 10^{2} – 3.09 x 10^{4}cfu/g) in all the samples compared with the total coliform counts. Statistically, there were no significant differences in the bacterial populations among the vegetables.

The identity and distribution of the bacterial isolates from the soil, water and vegetables obtained from the sites in Table 1, showed that all the isolates from the vegetables were derived from either soil or/and water.The following isolates displayed complete hemolysis on blood agar platesin Table 2;*Staphylococcus aurucularis, Yersinia pestis, Staphylococcus equorum, Enterobacter asburiae, Serratia fonticola, Photorhabdus asymbiotica, Providencia alcalifaciens, Plesiomonas shigelloides* and *Enterobacter cancerogenus*.*Staphylococcus xylosus, Proteus mirabilis, Salmonella enterica*(2), *Enterobacter gergoviae, Escherichia fergusonii, Escherichia coli, Enterobacter amnigenus* were all negative to the test (Table 2).EMB Isolates 5, 6, 7, 9 and 10 produced pink pigmentation after fermenting sorbitol, while EMB isolates 1, 2, 3, 4 and 8 were colorless after the fermentation of sorbitol.

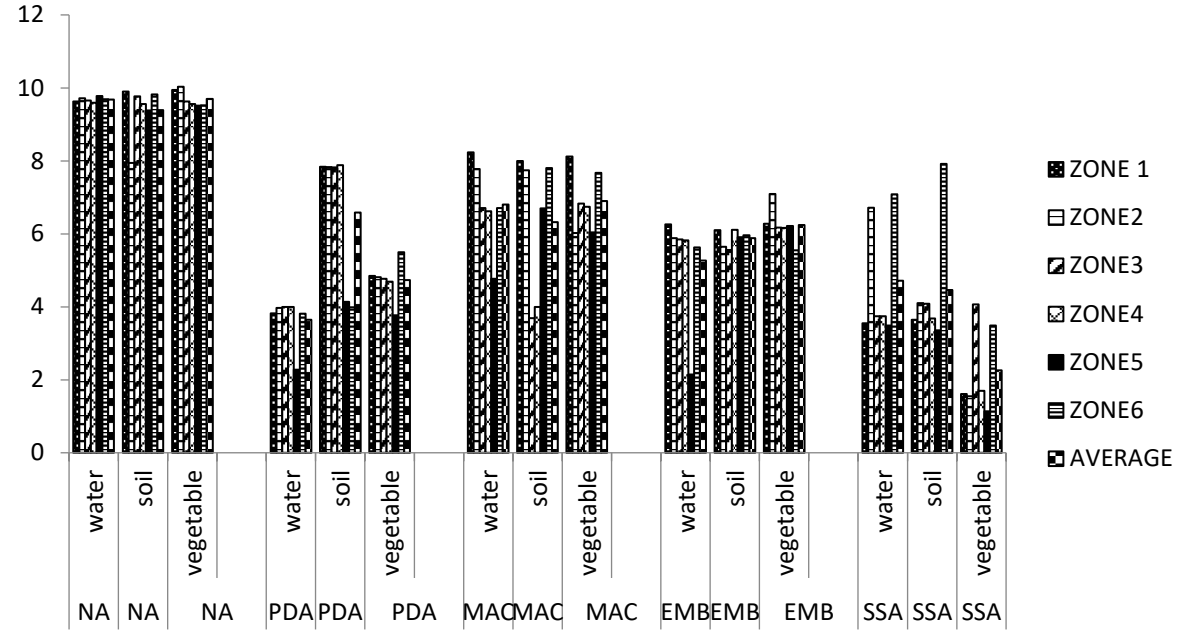

FIGURE 1: Microbial types and their population distribution in water, soils and vegetables from subsistence gardens in Lagos State

Legend: NA, Nutrient Agar; PDA, Potato Dextrose Agar; MAC, MacConkay Agar; EMB, Eosine methylene blue Agar; SSA, *Salmonella Shigella* Agar; Zone 1, Oko-Oba; Zone 2, IyanoIba; Zone 3, Ikorodu; Zone 4, Mushin; Zone5; Idi-Araba ; Zone 6, Epe.

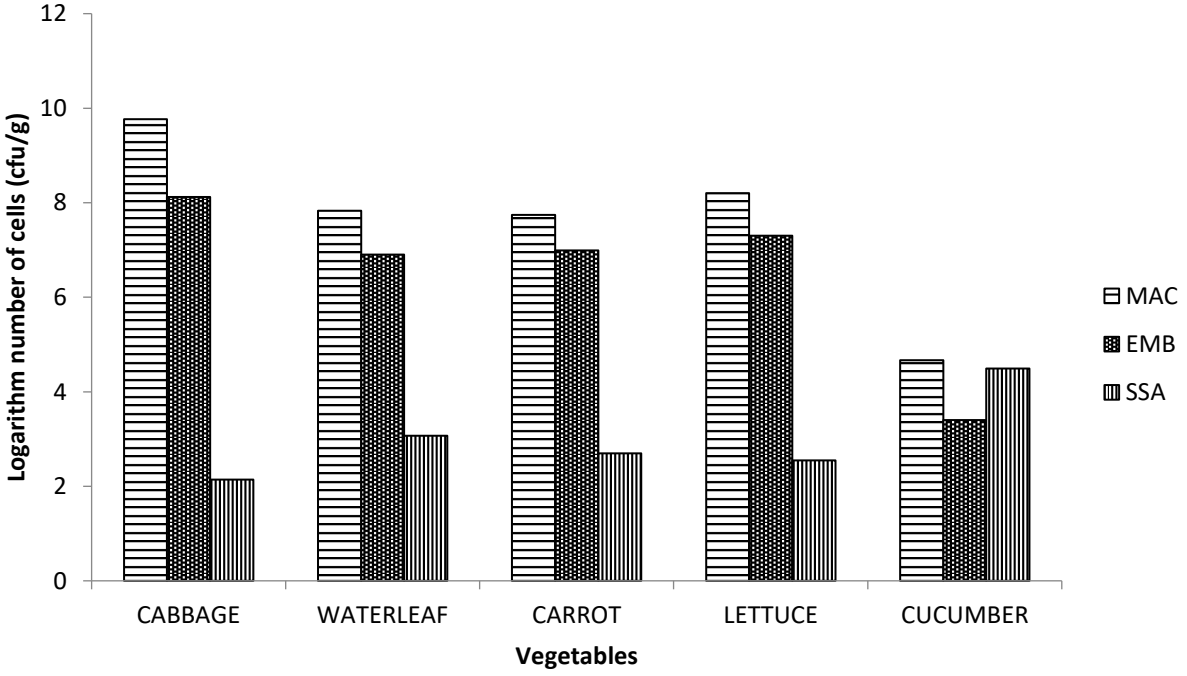

FIGURE 2: Population of coliforms in different vegetables from subsistence gardens in Lagos State
Legend: MAC, MacConkey Agar for the growth of total coliforms; EMB, Eosin methylene blue Agar for growthof fecal coliform; SSA, *SalmonellaShigella* Agar for growth of *Salmonella* and *Shigella* species.

TABLE 1: Isolates' distribution in water, soil and vegetables from subsistence gardens in Lagos State

ISOLATE CODE	ISOLATE IDENTITY	WATER	SOIL	VEGETABLE
EMB 1	*Staphylococcus aurucularis*	+	+	+
EMB 2	*Staphylococcus xylosus*	-	+	+
EMB 3	*Proteus mirabilis*	+	+	+
EMB 4	*Salmonella enterica*	-	+	+
EMB 5	*Yersinia pestis*	-	-	+
EMB 6	*Enterobacter gergoviae*	+	+	+
EMB 7	*Staphylococcus equorum*	+	+	+
EMB 8	*Enterobacter asburiae*	-	+	+
EMB 9	*Escherichia fergusonii*	+	+	+
EMB 10	*Escherichia coli*	+	+	+
SSA 11	*Salmonella enterica*	+	+	-
SSA 12	*Serratiafonticola*	+	+	+
SSA 13	*Photorhabdusasymbiotica*	-	+	-
SSA 14	*Providencia alcalifaciens*	+	-	-
SSA 15	*Plesiomonasshigelloides*	+	+	-
SSA 16	*Enterobacter cancerogenus*	+	+	-
SSA 17	*Enterobacter amnigenus*	+	+	-

TABLE 2: Hemolytic activities ofbacteriainwater, soil and vegetables from subsistence gardensin Lagos State

S/N	ISOLATE CODE	ISOLATE IDENTITY	HEAMOLYSIS
1	EMB 1	*Staphylococcus aurucularis*	+
2	EMB 2	*Staphylococcus xylosus*	-
3	EMB 3	*Proteus mirabilis*	-
4	EMB 4	*Salmonella enterica*	-
5	EMB 5	*Yersinia pestis*	+
6	EMB 6	*Enterobacter gergoviae*	-
7	EMB 7	*Staphylococcus equorum*	+
8	EMB 8	*Enterobacter asburiae*	+
9	EMB 9	*Escherichia fergusonii*	-
10	EMB 10	*Escherichia coli*	-
11	SSA 11	*Salmonella enterica*	-
12	SSA 12	*Serratiafonticola*	+
13	SSA 13	*Photorhabdusasymbiotica*	+
14	SSA 14	*Providencia alcalifaciens*	+
15	SSA 15	*Plesiomonasshigelloides*	+
16	SSA 16	*Enterobacter cancerogenus*	+
17	SSA 17	*Enterobacter amnigenus*	-

Legend: EMB, Eosine methylene blue Agar; SSA, *Salmonella Shigella* Agar; +, positive; -, negative.

Discussion

This study has shown thatcoliforms are abundant on the surfaces and within the tissues of vegetables. The coliform counts of the vegetables were high and far above the recommended standards for ready-to-eat vegetables which should be less than 10 coliform bacteria per gram (FAO, 1979). These high counts could be attributed to the unhygienic practices in the gardens, the water used in irrigation, soil and other environmental contaminations. Thehigh bacterial population in the vegetables werenot surprising since most often, the source of water for irrigation of the gardens in these communities are runoff waters and sewage water from domestic sources. High levels of bacteria and coliforms in vegetables have been repoted by Aliyu *et al.*(2005) and Seow *et al.* (2012). Peterside and Waribor (2006) reported that bacterial load on leafy vegetables increase with time during storage and exposure to contaminated water and soil.

The microbial populations in Figure 1 showed that on the average, the population of bacteria, total coliforms, fecal coliforms, fungi and *Salmonella-*

Shigella spp. were in the order 10^9, 10^6, 10^5, 10^3-10^6 and 10^3- 10^4cfu/g respectively. However, in some sites - Zone 2, 3 and 4 for vegetable and soil, and zone 5 for water samples respectively- the population counts on EMB agar were observed to be higher (between 10^5-10^7cfu/g and 10^7cfu/ml respectively) when compared to the counts on the MAC agar for total coliforms (10^3-10^6cfu/g). This abnormally could result from some of the constituents of the EMB agar favoring more luxuriant growth of fecal coliforms in samples from these sites.

Escherichia coli 0157:H7 was not isolated in this study. Although, some isolates appeared colourless after the fermentation of sorbitol, their identities were however, not of the genus *Escherichia*.Some Gram positive and Gram negative bacteria isolated were able to lyse erythrocyte. This property is based on the activity of most heterogeneous group of toxins called hemolysin. In recent years, the potential role of hemolysis has been demonstrated in various animal models and cell cultures (Bremm*et al.,* 1984). This ability to lyse blood cells could be a factor in determining pathogenicity of bacteria.

Most of the microorganisms that were observed on the vegetable surfaces are soil inhabitants. These organisms are members of a very large and diverse community of microbes that collectively are responsible for maintaining a dynamic ecological balance within most agricultural systems (Scheffer*et al.,* 1985). Vectors for disseminating these microbes include soil particles, airborne spores, and irrigation water which supports the distribution and prevalence observed in this study.

In conclusion, the increase in microbial loads on vegetables poses dangers to the consumers. This study revealed that vegetables are contaminated even before harvesting; therefore, it is advisable to properly sterilize vegetables before consumption either by steaming or by other means of sterilization. The contamination of vegetables can also be reduced by using irrigation water that meets the WHO standards of $\leq 10^3$ fecal coliform bacteria/100 ml (WHO, 1989) and by avoiding cross contamination that may arise as a result of close proximity to animals and waste dumps.

References

Aliyu, Y.U., Bassey, S.E. and Kawo, A.H. (2005). Bacteriological quality of vegetables sold in some shops around Kano metropolis, Nigeria. *Biological and Environmental Sciences Journal for the Tropics* 2(1): 145 – 148.

Bremm, K.D., König, W., Spur, B., Crea, A. and Galanos, C. (1984). Generation of slow-reacting substance (leukotrienes) by endotoxin and lipid A from human polymorphonuclear granulocytes. *Immunology*53(2):299–305.

Food and Agricultural Organization (FAO). (1979). Manuals of food Quality Control 4: Microbiological Analysis. FAO of the United Nations Publications, Rome, Italy. *FAO and Nutrition paper* 14(4): C11-C12.

Johnston, L.M., Jaykus, L.A., Moll, D., Anciso, J., Mora, B. and Moe, C.L. (2006) A field study of the microbiological quality of fresh produce of domestic and Mexican origin. *International Journal of Food Microbiology* 112:83-95.

Kim, J.K. and Harrison, M.A. (2008). Transfer of *Escherichia coli*0157:H7 to Romaine lettuce due to contact water from melting ice. *Journal of Food protection* 71(2): 252- 256.

Little, C.L. and Gillespie, I.A. (2008). Prepared salads and public health. *Journal of Applied Microbiology* 105: 1729- 1743.

McMahon, M.A.S. and Wilson, I.G. (2001). The occurrence of enteric pathogens and Aeromonas species in organic vegetables. *International Journal of Food Microbiology* 70(1-2):155-162.

Peterside, F.N.and Waribor, O. (2006). Bacteria associated with spoilage of fluted pumpkins leaves and their effect on the chlorophyll content. *Nigerian Journal of Microbiology* 20(1): 751- 756.

Seow, J., Ágoston, R., Phua, L. and Yuk, H. (2012). Microbiological quality of fresh vegetables and fruits sold in Singapore. *Food Control* 25(1): 39-44.

Scheffer, J., König, W., Hacker, J., Goebel, W. (1985). Bacterial adherence and hemolysin production from *Escherichia coli* induces histamine and leukotriene release from various cells. *Infection and Immunity* 50(1): 271-278

Web Resources

Society for General Microbiology (SGM) (2007, September 7). Lettuce, Leafy Greens and *E. Coli*. *Science Daily*. www.sciencedaily.com/releases/2007/09/070902193834.htm Retrieved on 23/08/2015.

New South Wales (NSW) Food Authority. (2007). Microbiological quality of fresh cut vegetables.NSW/FA/CP019/0903. http://www.foodauthority.nsw.gov.au/ Documents/corporate_pdf/microbiological-quality-fresh-leaves-CP019-0903.pdfRetrieved on 23/08/2015.

World Health Organization (WHO) (1989). Standard for horticulture or vegetable garden water ($\leq 10^3$ faecal coliform bacteria/100 ml).http://www.who.int/water_sanitation_health/dwq/whoiwa/en/Retrieved on 17/11/2015.