Bacteriological Study Of Vegetables From Markets Of Calabar Cross-River State Southeastern Nigeria.

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Citation

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Abstract

Background: Five vegetables, Lycopersicon esculentum (tomato), Allium cepa (onions), Capsicum annum (pepper), Telfairia occidentalis (fluted pumpkin leaves) and Cucumis sativus (Cucumber) were purchased from various markets in Calabar metropolis and studied to determine their level of bacterial contamination and safety for human consumption. Methodology: All the vegetables were washed with sterile water, cultured with blood agar and subcultured in nutrient agar to obtain pure cultures. Result: 153 bacteria isolates made up of seven species of bacteria were made from the five vegetable groups. Bacillus species 83(54.52%), Micrococcus spp 2(1.31%), Escherichia coli 36(23.53%), Proteus vulgaris 4(9.80%), Lactobacillus acidophilus 7(4.56%), Enterobacter aerogenes 15(9.80%) and Alcaligen faecalis 6(3.92%) were the bacteria species isolated. Bacillus species and E. coli were isolated from all the vegetables. All the seven bacteria species were isolated from tomatoes and fluted pumpkin leaves. Bacillus isolates were significantly higher than other bacteria isolates ($p<.05$). Viable bacteria counts from the vegetables were highest in fluted pumpkin leaves, $9.3x10^7$ cfu/ml, followed by that of Cucumber $9.0x10^7$ cfu/ml and the least count from pepper $1.8x10^7$ cfu/ml. Conclusion: Vegetable contamination with bacteria was observed in all the three markets; highest in Watt market and least in Marian market but statistically not significant ($P>0.05$). Poor processing by retailers and handling by sellers with unwashed hands was the attributed source of vegetable contamination with bacteria. Raw vegetables from the markets were considered unfit for human consumption and adequate cooking with proper handling before consumption is suggested.

BACKGROUND

Vegetables are those plants which are consumed in relatively small quantities as a side-dish or as a relish with staple food[5]. In order to keep them from losing their full turgidity, harvested vegetables often require low temperature and moist environment during storage[4]. As a result, vegetable in the form of leaves are often sprinkled with water after harvesting and during exposure in the open market. The moist condition of the vegetables fostered by the sprinkling of water together with low temperature condition encourages the growth of microorganisms[6]. These conditions also facilitate direct contamination by microorganisms through the handlers (buyers and sellers). Indirect contamination may also occur as a result of poor hygienic environment of the market. Consequently, harvested vegetables so soon begin to spoil if not sold immediately. However, cleaning processes reduce the number of microorganisms[6]. This study of bacteriological quality of vegetables was aimed at estimating the level of bacterial contamination of the vegetables commonly sold in different markets of Calabar metropolis and to determine the safety of the vegetables for human consumption.

MATERIALS AND METHODS

STUDY AREA

The vegetable samples numbering fifteen (five samples from each market) were bought, from three different markets namely: Akim, Marian and Watt, in Calabar metropolis. Calabar is located in the world map on coordinates $4\ 57^1$ N, $8\ 19^1$E in the eastern part of Nigeria with an estimated population of 1.2 million people[8]. South Eastern Nigeria has a tropical continental climate with distinct wet and dry seasons

HANDLING AND WASHING OF THE VEGETABLES

Sterile polythene bags were used to collect and transport the purchased samples to the Laboratory. A pair of forceps was flamed, allowed to cool, used to pick the samples and transferred into sterile containers. Sterile distilled water was

poured into these containers. Washing was done by shaking and rocking of the containers.

METHOD OF CULTURING THE WASHINGS FROM THE VEGETABLES

Serial dilution of the washings was done in which 1ml of each washing was added into a test-tube containing 9ml of sterile distilled water. Serial tenfold dilution was carried out from 10^{-1} to 10^{-10}. This was followed by pour plate technique. One millilitre aliquot of each dilution from 10^{-2}, 10^{-5} and 10^{-7} were cultured into Blood agar, MacConkey agar and Nutrient agar plates respectively. The lid was replaced in each case. Rocking of these plates were done as soon as the agar were poured, so as to have the microorganisms evenly separated during growth. Solidification of these agars was followed by incubation at 37C for 24hrs. After 24hrs, colonies were observed, counted and recorded.

SUB CULTURING OF THE CULTURES

The colonies were purified by sub culturing then in fresh nutrient agar plates. After purification, the isolates were maintained using nutrient agar slant and were kept in the refrigerator at 4C for identification.

IDENTIFICATION OF VARIOUS ISOLATES OBTAINED IN THE CULTURES

The following biochemical tests were carried out for the characterization and identification of the organisms, viz: Gram's stain, Catalase test, Indole test, Oxidase test, Sugar fermentation test, Motility test, Coagulase test, Citrate Utilization test, Hydrogen sulphide production test, Voges-Proskauer test, Methyl red test and haemolytic reaction on blood agar.

ESTIMATION OF INTENSITY OF CONTAMINATION

Intensity of contamination was estimated by using the viable count technique. Only plates that showed not less than 30 colonies and not more than 300 colonies were used for this estimation. Colony forming unit (CFU) was used as the standard unit for the estimation and is defined by:

Figure 1

$$\frac{1}{\text{CFU= Average colony count x dilution factor x Volume used.}}$$

RESULTS

The bacteria isolates from the vegetables in Akim market are shown in table 1. Altogether 53 bacteria isolates were made. Bacillus spp had the highest incidence 34(65.30%) followed by Escherichia coli with 10(20.00%), Lactobacillus acidophilus, Micrococcus spp and Proteus vulgaris recorded the lowest incidence of 1(1.60%) each, while Alcaligen faecalis had an incidence of 2(3.20%).

The bacteria isolates from the vegetable samples from Marian market are shown in table 2. Altogether 52 isolates were made. Again Bacillus spp had the highest incidence 24(45.50%) followed by E.coli 16(30.20%), E. aerogenes 7(14.00%), L. acidophilus 3(5.80%) while P.vulgaris and A.faecalis had the lowest incidence of 1(2.25%) each.

From the Watt market, 48 bacteria isolates were made (table 3). Bacillus spp had the highest incidence of 25(53.50%), followed by Escherichia coli 10(22.00%), Enterobacter aerogenes 4(7.20%), Lactobacillus acidophilus and Alcaligen faecalis 3(6.55%) respectively and Micrococcus spp 1(1.80%).

Table 4 shows a comparison of the bacteria isolates from the three markets of Calabar metropolis. From total of 153 bacteria isolates made from the three markets, 53(34.64%) were from Akim market, 52(33.99%) were from Marian market and 48(31.37%) were Watt market. Although the isolates from Akim and Marian markets were slightly higher than that of Watt market, they were not significantly higher ($P>0.05$) than that of the Watt market.

Of the seven bacteria species namely Bacillus spp, Micrococcus spp, Escherichia coli, Proteus vulgaris, Enterobacter aerogenes, Lactobacillus acidophilus and Alcaligen faecalis, from the three markets, Bacillus species isolates 83(54.25%) were significantly higher ($P<0.05$) than other bacteria isolates. In the order of their significance, Bacillus was followed by E. coli 36(23.53%) and Enterobacter aerogenes 15(9.80%). Others were Lactobacillus acidophilus 7(4.56%) Alcaligen faecalis 6(3.92%), Proteus vulgaris 4(2.61%) and Micrococcus species 2(1.31%).

The distribution of individual bacteria isolates on the vegetables is shown in table 5. Of the seven bacteria species, Bacillus species and Eschericha coli were isolated from all the five vegetable groups studied. The highest number of Bacillus isolates 31(37.75%) were from fluted pumpkin leaves while the least 3(3.61%) were from pepper. Also the highest number of E. coli isolates 14(38.8%) were from fluted pumpkin leaves and least 1(2.78%) were from pepper.

One isolate each of Micrococcus spp was made from tomatoes and fluted pumpkin. Proteus vulgaris was not isolated from onions and pepper, it has the highest isolate 2(50.0%) from tomatoes and 1(25.0%) from cucumber and pumpkin leaves respectively. Enterobacter acrogens and Alcaligen faecalis were also not isolated from onions and pepper. The highest number of E. aerogenes isolates 8(42.86%) were from fluted pumpkin leaves and the least 3(20.0%) from tomatoes. Also the highest member of A. alcaligen isolate 3(50.0%) were from fluted pumpkin leaves and the least 1(16.67%) was from tomatoes. Lactobacillus acidophilus was not isolated from pepper only. The highest number of L. acidophilus isolates 3(50.0%) were from fluted pumpkin leaves and least number 1(14.29%) were from cucumber and onions. With the exception of P. vulgaris with its highest number of isolates from tomatoes, five bacteria species namely Bacillus spp, E. coli, E. aerogenes, L. acidophilus and A. alcaligens had their highest isolates from fluted pumpkin leaves. An equal number of isolates of Micrococcus species was isolated from tomatoes and fluted pumpkin leaves (one isolate from each). All the seven bacteria isolates were made from fluted pumpkin leaves and tomatoes while only Bacillus spp and E. coli were found in pepper. Onions has only 3 bacteria isolates viz. Bacillus spp, E. coli and L. acidophilus. Cucumber has all the six bacteria isolates with the exception of Micrococcus species.

The percentage distribution of the isolates on the vegetables is represented in figure 1. Of the 153 bacteria isolates, 61(39.87%) were from fluted pumpkin leaves, 42(27.45%) from cucumber, 38(24.84%) from tomatoes, 8(5.23%) from onions and 4(2.61%) from pepper. The distribution of the isolates on the different vegetable was statistically significant ($P<0.05$). Fluted pumpkin had the highest number of isolates followed by cucumber, tomatoes, onions and pepper.

The viable bacteria counts from the different vegetables bought from markets of Calabar metropolis were shown in figure 2. Telfairia occidentalis (fluted pumpkin) recorded very high bacteria counts in all the three markets in Calabar metropolis with $5.0x10^7$ cfu/ml, $6.2x10^7$ cfu/ml and $9.3x10^7$ cfu/ml respectively in Akim, Marian and Watt Markets. Cucumis sativus (cucumber) recorded its highest count of $9.0x10^7$ cfu/ml in Watt market while Lycopersicon esculentum (tomatoes) had its highest count of $4.8x10^7$ cfu/ml in Akim market. Allium cepa (onions) recorded its highest bacteria count $3.0x10^7$ cfu/ml in Marian market while Capsicum annuum (pepper) had the highest bacteria count $1.8x10^7$ cfu/ml in Marian market.

Figure 2

TABLE 1: BACTERIA ISOLATES FROM AKIM MARKET SAMPLES

	ISOLATES	NUMBER OF ISOLATES	PERCENTAGE INCIDENCE (%)
1.	*Bacillus* spp.	34	65.30
2.	*Micrococcus* spp	1	1.60
3.	*Escherichia coli*	10	20.00
4.	*Proteus vulgaris*	1	1.60
5.	*Enterobacter aerogenes*	4	6.70
6.	*Lactobacillus acidophilus*	1	1.60
7	*Alcaligen faecalis*	2	3.20
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	Total	53	100.00

Figure 3

TABLE 2: BACTERIA ISOLATES FROM MARAIN MARKET SAMPLES.

	ISOLATES	NUMBER OF ISOLATES	PERCENTAGE INCIDENCE (%)
1.	*Bacillus* spp.	24	45.50
2.	*Escherichia coli*	16	30.20
3.	*Proteus vulgaris*	1	2.25
4.	*Enterobacter aerogenes*	7	14.00
5.	*Lactobacillus acidophilus*	3	5.80
6	*Alcaligen faecalis*	1	2.25
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	Total	52	100.00

Figure 4

TABLE 3: BACTERIA ISOLATES FROM WATT MARKET SAMPLES.

	ISOLATES	NUMBER OF ISOLATES	PERCENTAGE INCIDENCE (%)
1.	*Bacillus* spp.	25	53.50
2.	*Micrococcus* spp	1	1.80
3.	*Escherichia coli*	10	22.00
4.	*Proteus vulgaris*	2	3.20
5.	*Enterobacter aerogenes*	4	7.20
6.	*Lactobacillus acidophilus*	3	6.55
7	*Alcaligen faecalis*	3	6.55
		---	---
	Total	48	100.00

Figure 5

TABLE 4: COMPARISON OF BACTERIA ISOLATES FROM THE MARKETS IN CALABAR METROPOLIS

	Number of Isolates from the three markets				
BACTERIA ISOLATES	**AKIM**	**MARIAN**	**WATT**	**TOTAL**	**%**
Bacillus spp.	34	24	25	83	54.25
Micrococcus spp.	1	0	1	2	1.31
Escherichia coli	10	16	10	36	23.53
Proteus vulganis	1	1	2	4	2.61
Enterobater aerogenes	4	7	4	15	9.80
Lactbacillus acidophitus	1	3	3	7	4.56
Alcaligen faecalis	2	1	3	6	3.92
Total	**53**	**52**	**48**	**153**	**100**
%	**34.64**	**33.99**	**31.37**	**100**	

Figure 6

TABLE 5: THE NUMBER AND PERCENTAGE DISTRIBUTION OF INDIVIDUAL BACTERIA SPECIES ON THE FIVE DIFFERENT VEGETABLES

		Number and percentage (%) distribution on the vegetables				
BACTERIA SPECIES	**TOTAL NO OF ISOLATES**	**TOMATOES**	**CUCUMBER**	**ONIONS**	**PUMPKIN LEAVES**	**PEPPER**
Bacillus spp.	83	20 (24.10)	24 (28.92)	5 (6.02)	31 (37.35)	3 (3.61)
Micrococcus spp.	2	1 (50.0)	0	0	1 (50.0)	0
Escherichia coli	36	9 (25.0)	10 (27.78)	2 (5.56)	14 (38.89)	1 (2.78)
Proteus vulganis	4	2 (50.0)	1 (25.0)	0	1 (25.0)	0
Enterobater aerogenes	15	3 (20.0)	4 (26.67)	0	8 (42.86)	0
Lactbacillus acidophitus	7	2 (28.57)	1 (14.29)	1 (14.29)	3 (42.86)	0
Alcaligen faecalis	6	1 (16.67)	2 (33.33)	0	3 (50.0)	0
Total	**153**	**38** (24.84)	**42** (27.45)	**8** (5.23)	**61** (39.87)	**4** (2.61)

Figure 7

FIGURE 1: PERCENTAGE FREQUENCY DISTRIBUTION OF THE BACTERIA ISOLATES ON THE VEGETABLES.

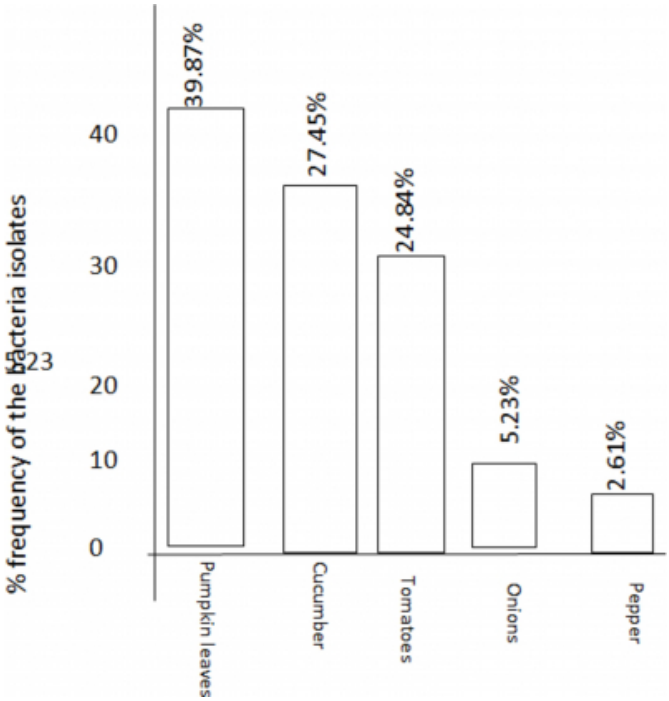
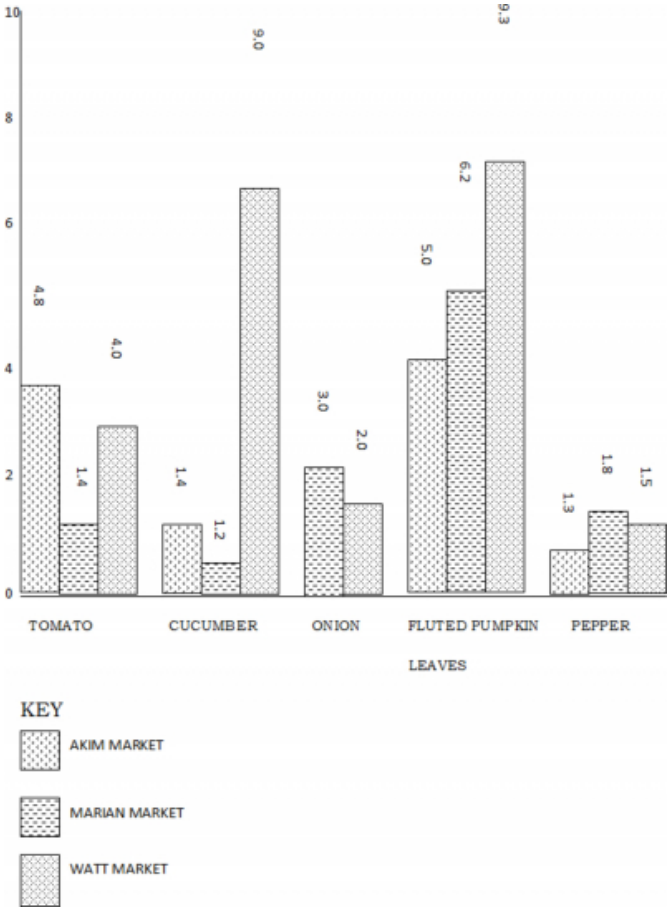


Figure 8

FIGURE 2: SHOWING VIABLE BACTERIA COUNTS (cfu/ml) FROM THE VEGETABLES



DISCUSSION

In Calabar Markets, the commonly sold vegetables such as Lycopersicum esculentum, Telfairia occidentalis, cucumis sativus, Allium cepa and Capsicum annuum were found to be contaminated with bacteria. This contamination could be as a result of poor processing methods which may involve washing with faecal contaminated stream water and spraying the vegetable with water. Though spraying with water gives fresh appearance to the vegetables and delays decomposition but it could add microorganisms, especially, psychrotrophs from water or ice[3]. This spray method provides moist surface that encourages bacteria growth on longer storage. Also vegetables are subject to contamination by containers unless adequately sanitized[2].

The bacteria isolates from the vegetable samples were Bacillus species, Micrococcus species, Escherichia coli, Proteus vulgaris, Enterobacter aerogenes, Lactobacillus acidophilus and Alcaligen faecalis. The distribution of these isolates varied with different vegetables. Bacillus species was found to have high count in Telfairia occidentalis while

low count was recorded in Capsicum annuum. Escherichia coli ranked next to Bacillus spp in high count and it was recorded in T. occidentalis. Micrococcus species was the isolate with the least count. The low bacteria count in Allium cepa and Capsicum annuum may be due to anti-microbial compounds in them which are inhibitory to multiplication of microorganisms[7]. Distribution of bacteria isolates also differed in the different markets. Akim market recorded the highest bacteria count in Bacillus species while Marian market recorded the least count in this isolate. No isolate of Micrococcus was found in Marian market samples but Akim and Watt markets yielded very low count for micrococcus species.

The higher incidences of Bacillus species over others may be due to its spores which could resist killing by high temperature of ultra-violet sun rays which may kill and reduce the bacteria load in vegetables during exposure and display for sale. High incidence of Escherichia Coli may probably be due to handling by buyers and sellers whose hands may have been contaminated with faecal matters or they may have been contaminated from the farm yards when fertilized with human and animal manures. Bacillus species has been implicated as one of the common contaminants of vegetables[1]. The presence of Enterobacter aerogenes and Alcaligen faecalis could probably be due to washing of these vegetables with water contaminated with faecal matter. Low incidences of Lactobacillus acidophilus may be attributed to their inability to produce spores like Bacillus species. Being acidophilic, their survival and multiplication require acidic and relatively anaerobic conditions, which are not provided for on these vegetables.

All the bacteria isolated were opportunistic pathogens, in that; they usually cause infection if suitable opportunity arises. This suitable opportunity could be seen in a person with weak natural immunity probably due to poor health, malnutrition, infection with HIV or drug therapy.

CONCLUSION

Adequate care should be taken in processing these vegetables to destroy the microorganisms before they enter inside the human body and obtain favourable conditions which could support their increase in number; thereby causing food-borne disease or food poisoning to the detriment of the human health.

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