Occurrence of Enterohaemorrhagic *Escherichia coli* O157:H7 in Vegetables Sold in Jos Metropolis, Nigeria

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Abstract: Vegetables contaminated with pathogens can cause food poisoning, and can serve as vehicles for transmission of *Escherichia coli* O157:H7 to humans. This study was therefore carried out to screen for the presence of O157:H7 strain of enterohaemorrhagic *Escherichia coli* in vegetables eaten raw and sold at the various vegetable markets in Jos metropolis. A total of 80 samples of vegetables namely; cucumber (*Cucumis sativus*), lettuce (*Lectuca sativa*), tomatoes (*Solanum lycopersicon*) and cabbage (*Brassica oleracae*) were collected from popular vegetable markets in Jos metropolis and analyzed. All vegetables were contaminated with *Escherichia coli* were isolated from 13.8% (11/80) of the vegetable samples, 25% (5/20) of cucumber had the highest contamination while tomatoes had the lowest contamination of about 5% (1/20). *Escherichia coli* O157:H7 (18.2%) was detected among the *Escherichia coli* isolates. All the *Escherichia coli* O157:H7 isolates were detected in cucumber thus accounting for 40.0% (2/5) of the *Escherichia* coli-positive cucumber samples. The presence of *Escherichia coli* O157:H7 in these vegetables poses public health concern and calls for improved hygienic practices in vegetable production and handling because these vegetables receive little or no heat treatment before consumption.
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Introduction

Escherichia coli O157:H7 has been implicated in a growing number of food-borne illnesses associated with the consumption of fresh products (Kasseborg *et al.,* 2004). In 1982, there were three outbreaks of haemorrhagic colitis caused by *E. coli* serotype O157:H7 in Oregon, Michigan and Ontario (Canada). Two common-source outbreaks which were probably food-related were responsible for 66 cases of haemorrhagic colitis, 12 cases of haemolytic uremic syndrome (HUS), and 17 deaths (Carter *et al.,* 1987). *E. coli* was responsible for haemorrhagic colitis during outbreaks of bloody diarrhea in Michigan and Oregon, USA (Schlundt, 2001). The bacteria has been epidemiologically linked to the consumption of ground beef (Schlundt, 2001). It has also been found to be strongly associated with lettuce, undercooked beef (Davis *et al.,* 2005). Several outbreaks of *E. coli* O157:H7 infection have been reported in the United States and other developed as well as developing nations (Al-Dragy and Baqer, 2014).

Escherichia coli O157:H7 is transmitted by foods contaminated by animal and human faecal matters. Besides undercooked ground beef which has been implicated as the leading food vehicle for the transmission of *E. coli* O157, raw vegetables used as salad are also becoming increasingly reported as important means of transmission (Beuchat, 1999). Different researchers attributed outbreaks of *E. coli* O157:H7 infections to contaminated lettuce (Achers *et al.,* 1996), raddish sprout (Michino *et al.,* 1990), Alfalfa sprout (Taormina *et al.,* 1999) and Potatoes (Chapman *et al.,* 1997).

The contamination of salad vegetables by *E. coli* O157:H7 could occur at various stages ranging from cultivation to dining table. Therefore, vegetables that are cultivated in soil fertilized by night soil and farm animal manure are likely to be contaminated with *E. coli* O157 (Islam *et al.,* 2004; Solomon *et al.,* 2002; Ingham *et al.,* 2004).

Vegetables used for preparing salads could serve as possible route of transmission of *E. coli* O157 if they were grown in soil fertilized by either human wastes or animal manure. There is also the possibility of contamination of salad vegetables with *E. coli* O157 by hands of vegetable handlers (Medeiros *et al.,* 2001; Mnkeni *et al.,* 2006). In addition to the aforementioned routes of transmission which are all prevalent in the study area, sewage and wastewater were also used for irrigation of vegetables by vegetable farmers. Yet there is no data on *E. coli* O157 from raw vegetables that are frequently consumed as salad in the study area. This study therefore was aimed at assessing the bacteriological quality of some salad vegetables with respect to occurrence of *E. coli* O157:H7.

Materials and Methods

Sample collection

A total of 80 salad vegetable samples were collected during the period of July to December, 2014. Twenty (20) samples each of tomatoes, cucumber, cabbage, and lettuce were collected from vegetable markets at Farin-Dada, Angwan Rukuba, Terminus and Chobe. The samples were collected in clean plastic bags and transported in ice-pack to the laboratory at Department of Microbiology, Faculty of Natural Sciences, University of Jos, Jos, Nigeria.

Total bacterial count and coliform count

Ten gram (10g) of each sample was shredded and homogenized in 90 ml of single strength buffered peptone water to yield 10^{-1} stock. The stock was diluted further to 10^{-5}. Aliquot (0.1ml) of the 10^{-3} suspension was spread on nutrient agar plates and eosin methylene blue (EMB) agar in duplicate and incubated at 37°C for 24 hours. The colonies on both media were counted and expressed in cfu/g.

Isolation of *Escherichia coli* O157:H7

The stock suspension (10^{-1}) of the vegetables made by homogenizing 10g of each vegetable in 90ml of modified tryptic soy broth (mTSB) supplemented with vancomycin (4ml/g) and incubated at 37°C for 24 hours (Sanderson *et al.*, 1995). A loopful of the enriched samples were plated on sorbitol MacConkey agar (SMA, Oxoid) supplemented with ceftixime to test for non-sorbitol fermenting bacteria (colourless colonies).

Non-sorbitol fermenting colonies were sub-cultured on nutrient agar slants and incubated for 24 hours at 37°C. This was kept in the refrigerator for further analyses. The colonies were tested for oxidase activity; all oxidase-negative colonies were biochemically confirmed to be *Escherichia coli* using indole test, citrate utilization test (Simmons citrate agar), methyl red and Voges-Proskauer test (MR-VP). Isolates identified to be *E. coli* were serologically using *E. coli* O157:H7 latex agglutination kit (Oxoid) following manufacturer's instruction.

Statistical analysis of data

Data generated from this study were analysed using Statistical Package for the Social Sciences (SPSS) version 21. One-way analysis of variance (One-Way ANOVA) was used to compare the mean total bacteria and coliform counts from the various vegetables as well as vegetable markets. Level of significance (p-value) was set to be 0.05. Therefore, $p \leq 0.05$ was considered to be statistically significant. The mean total bacteria and coliform counts were presented in charts.

Results

Vegetables obtained from four different vegetable markets in Jos metropolis were analysed for total bacterial counts and coliform counts. Table 1 presents the distribution of total bacterial counts in the various vegetables based on locations. Mean total bacterial counts obtained from tomatoes was significantly different (F = 6.290; P = 0.002**). Tomato samples from Faringada had the highest mean bacterial counts (5.19 ± 0.05) followed by samples from Angwan Rukuba (5.02 ± 0.08) while tomato samples from Terminus had the least total bacterial counts (4.74 ± 0.04).

Similarly, comparison of mean bacterial counts obtained from cucumber samples from the four locations of Faringada, Angwan Rukuba, Terminus and Chobe were significantly different (F = 3.515; P = 0.029*). Samples from Faringada and Angwan Rukuba (5.37 ± 0.03 and 5.25 ± 0.06 respectively) had statistically equal bacterial counts. These counts were closely followed by counts of samples from Chobe (4.95 ± 0.09) while the least mean bacterial counts were obtained from Terminus (3.99 ± 0.50).

Cabbage samples from Angwan Rukuba had the highest mean total bacterial counts (5.15 ± 0.02) which was significantly different (F = 3.679; P = 0.025*) from the counts obtained from Faringada (4.67 ± 0.15), Terminus (4.74 ± 0.09) and Chobe (4.61 ± 0.11). On the other hand, lettuce samples obtained from all the four locations showed no significant difference (F = 0.488; P = 0.694).

Table 2 depicts the distribution and comparison of mean coliform counts in vegetables based on locations. The comparison showed that there were significant difference ($P < 0.05$) in the mean coliform counts of tomatoes (F = 4.851; P = 0.008**), cucumber (F = 3.290; P = 0.036*) and cabbage (F = 3.105; P = 0.044*) collected from the four locations. There was no significant difference ($P > 0.05$) in the mean coliform count of lettuce (F = 0.265; P = 0.850). A cursory analysis of the result revealed that tomato samples obtained from Faringada had the highest level of coliform (5.12 ± 0.07) followed by Terminus (4.73 ± 0.05) and Chobe (4.84 ± 0.11) had statistically equal mean coliform counts.

In the same vein, mean coliform count of cucumber indicated that samples from Faringada had the highest level of contamination (5.38 ± 0.03) followed by samples from Angwan Rukuba (5.03 ± 0.49) and Chobe (4.90 ± 0.09) with samples obtained from Terminus (3.96 ± 0.49) having the least mean coliform count. Though there was significant difference (P = 0.044) in the mean coliform count of cabbage, samples obtained from Angwan Rukuba (5.15 ± 0.02) and Terminus (4.74 ± 0.09) had the highest count; these were closely followed by mean coliform counts of cabbage samples collected from Chobe (4.61 ± 0.11). Samples from Faringada had the least (3.92 ± 0.74) counts.

Table 1: Distribution and comparison of total bacterial counts in vegetables based on locations

Location	**No. of samples**	**Mean ± SEM (Log_{10} CFU/g)**			
		Tomatoes (n=20)	**Cucumber (n=20)**	**Cabbage (n=20)**	**Lettuce (n=20)**
Faringada	20	5.19 ± 0.05^{a}	5.37 ± 0.03^{a}	4.67 ± 0.15^{b}	4.11 ± 0.79
Angwan Rukuba	20	5.02 ± 0.08^{ab}	5.25 ± 0.06^{a}	5.15 ± 0.02^{a}	4.34 ± 0.84
Terminus	20	4.74 ± 0.04^{c}	3.99 ± 0.50^{b}	4.74 ± 0.09^{b}	4.74 ± 0.04
Chobe	20	4.92 ± 0.09^{bc}	4.95 ± 0.09^{ab}	4.61 ± 0.11^{b}	4.59 ± 0.11
Total	80	4.92 ± 0.04	4.75 ± 0.19	4.75 ± 0.06	4.52 ± 0.18
ANOVA		6.290	3.515	3.679	0.488
P-value		0.002^{**}	0.029^{*}	0.025*	0.694

Values are means of multiple readings. Mean values were separated by Duncan's multiple range test. Values with different superscripts in the same column are significantly different.
* = significant difference exists at $P \leq 0.05$
** = significant difference exists at $P \leq 0.01$

Table 2: Distribution and comparison of mean coliform counts in vegetables based on locations

Location	**No. of samples**	**Mean ± SEM (Log_{10} CFU/g)**			
		Tomatoes (n=20)	**Cucumber (n=20)**	**Cabbage (n=20)**	**Lettuce (n=20)**
Faringada	20	5.12 ± 0.07^{a}	5.38 ± 0.03^{a}	3.92 ± 0.74^{b}	4.41 ± 0.85
Angwan Rukuba	20	5.09 ± 0.03^{ab}	5.03 ± 0.08^{ab}	5.15 ± 0.02^{a}	4.28 ± 0.83
Terminus	20	4.73 ± 0.05^{c}	3.96 ± 0.49^{b}	4.74 ± 0.09^{a}	4.73 ± 0.05
Chobe	20	4.84 ± 0.11^{c}	4.90 ± 0.09^{ab}	4.61 ± 0.11^{ab}	4.39 ± 0.11
Total	80	4.89 ± 0.05	4.69 ± 0.19	4.75 ± 0.06	4.49 ± 0.19
ANOVA		4.851	3.290	3.105	0.265
P-value		0.008^{**}	0.036^{*}	0.044*	0.850

Values are means of multiple readings. Mean values were separated by Duncan's multiple range test. Values with different superscripts in the same column are significantly different.
* = significant difference exists at $P \leq 0.05$
** = significant difference exists at $P \leq 0.01$

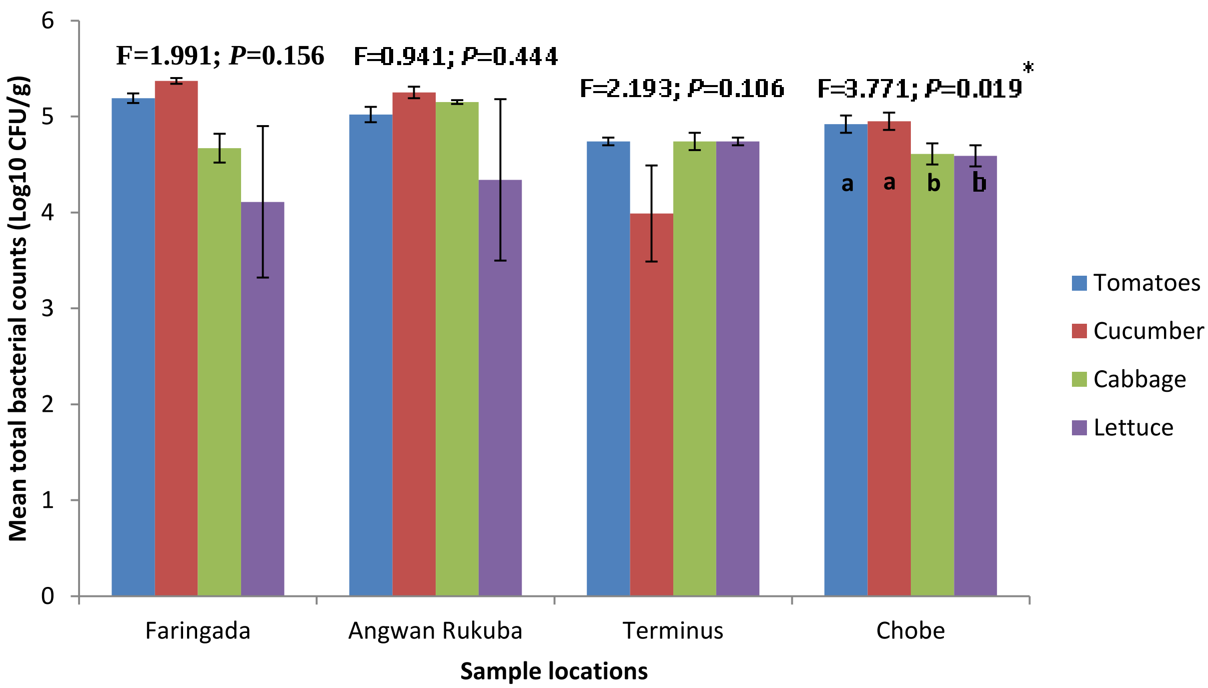

Figure 1: Mean total bacterial counts obtained from vegetables (F = analysis of variance, *P*= level of significance, * = significance difference exists at $P \leq 0.05$).

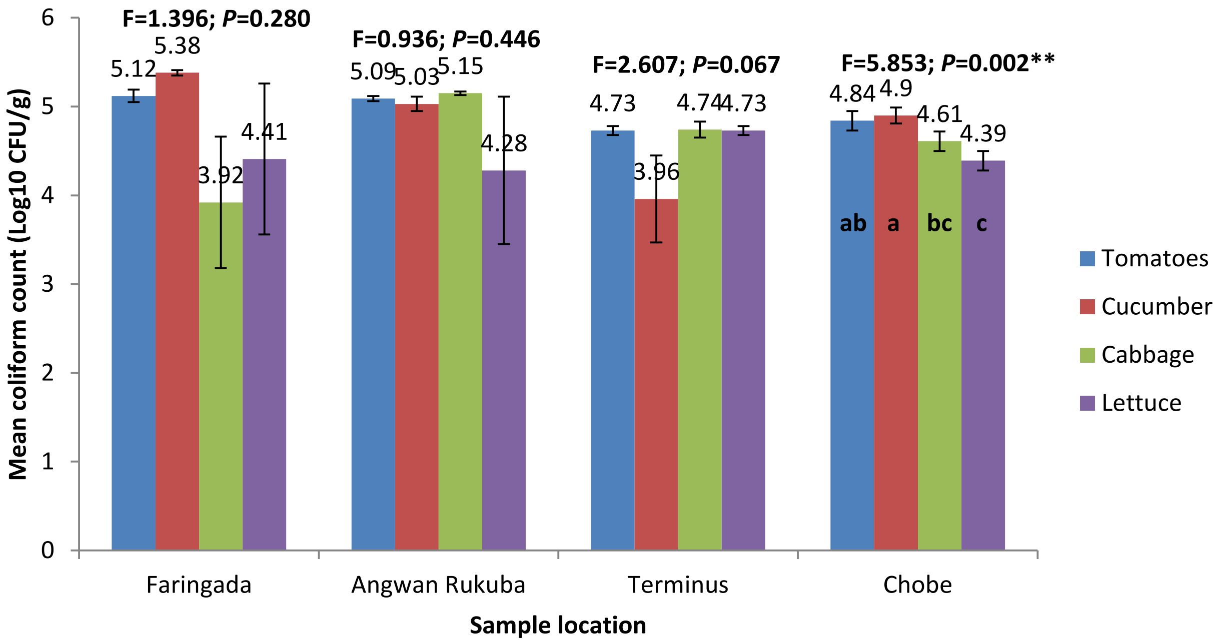

Figure 2: Mean coliform counts obtained from vegetables (F = analysis of variance, *P*= level of significance, ** = significance difference exists at $P \leq 0.01$).

Table 3: Distribution of *Escherichia coli* O157:H7 isolated from vegetables

Sample	No. of sample	No. positive for *E. coli* (%)	No. positive for *E. coli* O157:H7 (%)
Tomatoes	20	1(5.0)	0(0.0)
Cucumber	20	5(25.0)	2(40.0)
Cabbage	20	2(10.0)	0(0.0)
Lettuce	20	3(15.0)	0(0.0)
Total	80	11(13.8)	2(18.2)

Analysis of variance (ANOVA) was used to compare the mean total bacterial counts of the various vegetables obtained from each location and the results were presented in Figure 1. There was no significant difference ($P > 0.05$) in the mean total bacterial count of the vegetables in all the locations except for Chobe (F = 3.771; P = 0.019*). The results of samples from Chobe showed tomatoes and cucumber to have equal mean total bacterial counts which were significantly different ($P < 0.05$) from the mean total bacterial counts of cabbage and lettuce.

Similarly, vegetable samples from Faringada (F = 1.396; P = 0.280), Angwan Rukuba (F = 0.936; P = 0.446) and Terminus (F = 2.607; P = 0.067) showed no statistically significant difference ($P > 0.05$); but samples from Chobe showed significant difference (F = 5.853; P = 0.002**); cucumber had the highest mean coliform count followed by tomatoes while lettuce had the least mean coliform count (Figure 2).

Out of the 80 vegetable samples collected from the four markets, 13.8% (11/80) had *E. coli* contamination. Cucumber had the highest level of contamination, 5(25.0%), lettuce 3(15.0%), cabbage 2(10.0%) while tomatoes had the least contamination 1(5.0%). Further analysis showed that only cucumber had *Escherichia coli* O157:H7 with 40.0% (2/5) of the *E. coli* isolated from cucumber being of the O157:H7 strain (Table 3).

Discussions

Escherichia coli O157:H7 is the most studied strain among all the other pathogenic strains *Escherichia coli* because it has been recognised as the leading cause of human food borne infection throughout the world with fatal complications such as haemolytic uraemic syndrome that culminates in renal failure. This strain can be transmitted to humans by direct and indirect methods such as contaminated beef, ground meat, water, raw milk, fruit juice and vegetables. In this study *Escherichia coli* was detected in 18.2% vegetable samples eaten raw which was lower than those recorded by Enabulele and Uraih

(2009) from Nigeria. Though the rate of contamination was low, the facts remains that such contaminated vegetables serve as potent vehicles of *E. coli* O157:H7 infection. The low result obtained in this can be attributed to the differences in sample size, geographical area and agricultural method for vegetable production.

Escherichia coli is a normal flora of the digestive tract of both humans and animals and could contaminate the vegetables from different source either from animal manure used as soil fertilizer or through contaminated transportation vehicles. All the different types of vegetable sampled were contaminated with *Escherichia coli*, the highest percentage of *Escherichia coli* was from cucumber and lowest percentage was from tomatoes. The total bacteria count from all vegetables obtained from various vegetable market shows highest contamination in tomatoes and cucumber from Faringada market, Angwan Rukuba, Chobe and the least from Terminus market, cabbage obtained from Angwan Rukuba market had the highest contamination, while cabbage obtained from Terminus market was slightly more contaminated than cabbage obtained from Faringada and lowest contamination was in samples from Chobe, no significance difference in the level of contamination of lettuce obtained from the different markets was observed. This could be attributed to these markets having comparable level of hygiene. Similar studies in developed countries have been unsuccessful in isolating *E. coli* O157:H7 (Martinez *et al.*, 2000; Mukherjee *et al.*, 2004) owing to higher level of hygiene practices in developed countries compared to developing countries.

The mean coliform count of tomatoes obtained from the four various markets had the highest coliform mean count, the second highest in Angwan Rukuba market, terminus market and the least in chobe market, cucumber samples obtained from Angwan Rukuba had the highest mean coliform count from tomatoes obtained, second highest was from Faringada market, chobe and the lowest mean coliform count was in cucumber samples obtained from Terminus market. This may be due to poor handling of the vegetables by farmers or vegetable vendors. This result of this study was similar to the findings of Sharif and Arafa (2004) who found *Escherichia coli O*157:H7 in 11.7% of studied vegetables and Abongo (2008) who found prevalence of *Escherichia coli* O157:H7 ranging from 0% - 33% in onions and cabbage respectively. According to the current results and results of the previous studies, the presence of *Escherichia coli* O157:H7 in these vegetables calls for public health concern, because these vegetables receive little or no heat treatment before consumption; and one of the haemolytic uraemic syndrome risk group are children, pediatricians could contribute as prevention agents.

It is likely that humans and cattle can shed this pathogen in their faeces, this source of manure are not usually treated to remove pathogens before application to the soil in the farms. The presence of *Escherichia coli* O157:H7 in vegetables eaten raw therefore creates a potential health concern; this concern is heightened by the fact that these vegetables are used in combination to make salad which are consumed raw, secondly they are effective at low dose, which can be as low as 10 cells of *Escherichia coli* O157:H7 in a portion of food for immunocompromised people, children and the elderly (FDA, 1993).

Conclusion

This study showed that *Escherichia coli* O157:H7 was present in the vegetable samples in the study area; *Escherichia coli* O157:H7 was isolated from lettuce sample. Hence, there is the need for enlightenment of the public on adequate personal hygiene as well as vegetable handling to forestall outbreak of *Escherichia coli* O157:H7 infection in the study area.

Conflict Of Interest

No conflict of interest was declared.

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