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Potential Risk of Transmitting *Escherichia coli* O157:H7 through Some Vegetables Sold in Zaria Metropolis

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Abstract

This study was designed to assess the potential risk of transmitting *E. coli* O157:H7 through vegetables. Suspensions (25g of vegetable in 225ml of distilled water) of 100 vegetables, 20 each of spinach, carrot, cabbage, lettuce and tomatoes were separately cultured on Eosine Methylene Blue Agar (EMBA) for the viable count and isolation of *E. coli*. The isolates characterized biochemically as *E. coli* were sub-cultured on Sorbitol-MacConkey agar which is selective for *E. coli* O157:H7. The efficacy of salt and vinegar in disinfecting the vegetables was examined on the *E. coli* O157:H7 isolates. Various concentrations of vinegar and salt were tested against *E. coli* O157:H7 using agar well diffusion method and tube dilution technique. Highest *E. coli* count was found in lettuce (22.0×10^4 CFU/g) with carrot having the lowest *E. coli* count (8.0×10^4 CFU/g). The prevalence of *E. coli* O157:H7 was 10% in spinach, 5% in tomatoes and 0% each for cabbage, carrot and lettuce. The minimum inhibitory concentrations (MICs) were 12.5% (v/v) and 0.0125g/ml and the minimum bactericidal concentrations (MBCs) were 50% (v/v) and 0.025% g/ml, for vinegar and salt respectively. Vegetables were found to harbor pathogens like *E. coli* O157:H7. Salt and vinegar are good disinfecting agents in washing the vegetables.

Keywords: Potential, Risk, Transmitting, *E. coli*, Vegetables

INTRODUCTION

Vegetables are edible parts of plant which could be eating raw or undercooked (Asfah *et al*., 2013). Some vegetables can be consumed raw while some must be cooked to destroy toxins and microbes for safe consumption (Asfah *et al*., 2013). Vegetable tissue is rich in carbohydrates which serve as source of nutrients making them prone to microbial invasion (Adams and Moss, 1999). Vegetables serve as potential vehicles for transmission of human infections such as those caused by enteric microbes. Infection with *E. coli* O157:H7 follows ingestion of contaminated food or water, or oral contact with contaminated surfaces. It is highly virulent, with a low infectious dose (10 to 100 CFU). Bacteria can contaminate raw vegetables in several ways namely; through contaminated water used on farmland, direct contact with contaminated soil or during processing by the consumer (FDA, 2006). In Nigeria the vegetables such as carrot are sold by unlicensed vendors with poor hygiene (Beuchat, 1997). Carrot can become contaminated through contaminated soil, water, improper handling, packaging and transportation (Beuchat, 1997). The major pathogenic microorganisms associated with the carrot are viruses, bacteria, fungi, and parasite (Adams and Moss, 1999). Lettuce has been linked to numerous out break associated with *E. coli* O157:H7. Some of such outbreaks includes the cases reported from Northern Illnois and Connecticut USA (Elizabeth *et al.*, 1999), and the contamination is most likely through animal feces (Adams and Moss, 1999).

Spinach, tomato and cabbage were similarly incriminated in transmitting pathogens (Beuchat, 1997).

Escherichia coli live in both intestine of human and animals as normal flora, however some *E. coli* strains have developed the ability to cause disease of gastrointestinal, urinary or central nervous system in even the most robust human host (James and James, 1998).

Diarrhoegenic group of *E. coli* include; enterotoxigenic *E. coli* which caus 56 travelers' diarrhoea, enteroheamorrhagic *coli* which causes hemolytic-uremic syndrome and heamorrhagic colits, enteroaggregative *E. coli* which cause persistent diarrhea and enteropathogenic which causes watery infant diarrhea. There is one of its deadly strains known as *E. coli* O157:H7- entero-hemorrhagic *E. coli* (EHEC) which often comes from feces of cows, goat, and sheep (FDA, 2006). The pathogen has high resistance to antibiotic, as previously reported that an increased in an antibiotic resistance has been noted within *E. coli* O157:H7 over the last 20 years (Tserenpuntsag *et al*., 2005). Enterohaemorrhagic *E. coli* (EHEC) possess specific, plasmid coded fimbriae for adhesion to enterocytes. They can also produce prophage that determined cytotoxins (shiga-like toxins or verocytotoxins) (Hajmeer *et al*., 2006). Transmission of intestinal infections is usually indirect via food, drinking water, or surface water (Brooks *et al.*, 1995). *Escherichia coli* O157:H7 causes severe diarrhea and kidney damage (James and James, 1998). The source of transmission of ***E.*** *coli* O157:H7 is via infected cow feces used as animal manure on vegetable farms or watering of vegetables farm with contaminated sewage (Mahon *et al.*, 1997).

MATERIALS AND METHODS

Sample Collection and Handling

A total of one hundred vegetables, twenty each for tomatoes, spinach, lettuce, cabbage and carrot were collected from Samaru market in clean polythene bags. The samples were identified by the local sellers. The taxonomic identity of the samples was confirmed by a botanist in the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria.

Ten grams of each sample was weighed and macerated using sterile mortar and pestle. The macerated samples were each mixed with 90ml of sterile normal saline to form a stock suspension. Serial dilution of each stock suspension was carried out (10^1- $10^{5)}$. An aliquot of 0.1ml from the 10^3 and 10^5 ilutions were separately inoculated on osine Methylene Blue Agar (EMBA) sterile plates using spread plate method (in duplicate). These were incubated at 37^{o}C for 24 hours. Colonies were counted and characterized based on their Gram reactions and reactions to indole, methyl-red, Voges-Proskauer and citrate utilization tests (IMViC). The colonies characterized as *E. coli* were sub-cultured onto SMAC (sorbitol macconkey agar) and incubated at 37^{o}C for 24 hours. The colonies that appeared colorless were identified as *E. coli* O157: H7 (Ogden *et al*., 2001).

Antibacterial Effects of Salt and Vinegar on *E. coli* O157:H7

Stock salt solution of 20mg/ml was prepared and sterilized in autoclave at 121^{o}C for 15 minutes. Half fold serial dilution was carried out aseptically by mixing equal volume of the salt solution and distilled water. Various concentrations of the vinegar were also prepared by half-fold dilution (The stock solution was considered 100% v/v). The inoculum of the *E. coli* O157:H7 was standardized by matching the turbidity of the suspension of the test organism with 0.5 McFarland standard (prepared by adding 0.5ml of 1% barium chloride to 99.5ml of 1% sulphuric acid). The standardized inoculum of the *E. coli* O157:H7 was then tested against the pre-diluted salt concentrations of 10mg/ml, 5mg/ml, 2.5mg/ml and 1.25mg/ml; 100%, 50%, 25%, and 25% of vinegar for the susceptibility of the isolate, using agar well diffusion method.

Determination of MIC and MBC (Tube Dilution Method)

The stock solutions of both salt and vinegar, were serially diluted in Mueller-Hinton broth by half fold (5ml of each of salt and vinegar stock solutions in 5ml of Mueller-Hinton broth separately). The lowest concentrations (of salt and vinegar) that showed antibacterial activity (in the agar diffusion method above) were diluted further, as previously described. Therefore, lower solutions (0.625 mg/ml salt and 6.25% vinegar) were prepared and tested for MIC.

Then, 0.1ml of the standardized inoculum were added to the solutions and incubated at 37ºC for 24 hours. The tubes were examined for turbidity to determine the MIC. The ones without turbidity were sub-cultured on Mueller-Hinton agar plates and incubated over night at 37ºC to determine the MBC. The dispersion of the data recorded was measured using standard deviation.

RESULTS

Out of the 100 samples examined (20 samples each of the vegetables), 5(25%) from tomato, 2(10%) from carrot, 6(30%) from spinach, 4(20%) from cabbage and 3(15%) from lettuce were found to be positive for *E. coli*. The highest *E. coli* count of 2.2×10^5CFU/g was found in lettuce whereas; the lowest count was recorded in carrot with 8.0×10^4CFU/g. The average counts obtained from Tomato, Spinach and Cabbage were 1.5×10^5, 1.2×10^5 and 1.1×10^5CFU/g respectively. However, only three isolates of *E. coli* O157:H7 were identified, 1 (5%) from Tomato and 2 (10%) from Spinach.

Table 1: Occurrence of *Escherichia coli* O157:H7 in vegetable samples sold in Samaru market, Zaria.

Samples	**Number Analyzed**	**No. positive for *E. coli* (%)**	**Average *E.coli* count CFU/g**	**Occurrence of *E. coli* O157: H7 (%)**
Tomatoes	20	5(25)	15×10^4	1(5)
Cabbage	20	4(20)	11×10^4	0(0)
Carrot	20	2(10)	8×10^4	0(0)
Spinach	20	6(30)	12×10^4	2(10)
Lettuce	20	3(15)	22×10^4	0(0)
Total	100	20(20)	14×10^4	3(3)

Zones of inhibition were obtained, from all the concentrations of both salt and vinegar tested. However, the zone of inhibition increases with increase in concentration (Tables 2 and 3).

Table 2: Antibacterial Effect of Various Salt Concentrations against *E. coli* O157:H7

Key: SD indicates Standard Deviation

Concentration (mg/ml)	20	10	5	2.5
Zone of Inhibition ± SD (mm)	15±2.7	13±2.7	10±2.7	8±2.7

Table 3: Antibacterial Effect of Various Vinegar Concentrations against *E. coli* O157:H7

Concentration % (v/v)	100	50	25	12.5
Zone of Inhibition ± SD (mm)	19±3.35	16±3.35	13±3.35	10±3.35

Key: SD indicates Standard Deviation.

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of salt (g/ml) against *E. coli* O157:H7 were 0.025 and 0.1 respectively. The MIC and MBC of vinegar were 12.5 and 25% respectively (Tables 4 and 5).

Table 4: Minimum inhibitory concentration (MIC) of salt and vinegar against *E. coli* O157:H7

Test organism	Vinegar (%v/v)			Salt (mg/ml)		
	25	12.5	6.25	5	2.5	1.25
E. coli O157:H7	+	+*	-	+	+*	-

Key: + = indicates activity; - = indicates no activity; +* indicates MIC.

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Table 5: Minimum Bactericidal Concent of Salt and Vinegar against *E. coli* O157:H7

Test organism	Vinegar (v/v) %				Salt (mg/ml)			
	100	50	25	12.5	20	10	5	2.5
E .coli O157:H7	+	+	+*	-	+	+*	-	-

Key: + = indicates activity; - = indicates no activity; +* indicates MBC

DISCUSSION

In the current study, *E. coli* count was greater than 100CFU/g which is above the standard for vegetables consumption (FDA, 2006) and the implicated vegetables are therefore not safe for consumption. The relatively lower prevalence and lower *E. coli* count in both Tomato and Carrot may be attributed to their fruity nature that may be less prone to persistent bacterial contamination (Beuchat and Ryu, 1997). Cabbage, Lettuce and Spinach are leafy with larger surface area for exposure to microbial contamination making them to have both higher count and prevalence of *E. coli* (Beuchat and Ryu, 1997). The major sources of contamination for vegetables are human or animal feces (Wang and Doyle, 1998). Outbreak associated with eating of contaminated vegetables caused by *Escherichia coli* have been reported during the last few years (Wang and Doyle, 1998). Centre for disease for control claim that 3% of food borne illnesses is traceable to farm but 97% are due to contamination on field. Several types of vegetables served as vehicle for transmitting bacteria especially *Escherichia coli (*Asfah *et al*., 2013). The prevalence of *Escherichia coli* O157:H7 in the vegetables sample was lower than the previous reports (Aborgo *et al*, 2008; Benard, 2008; Stephen and Nduka 2009 and Reuben and Makut 2014), who reported the prevalence of *Escherichia coli* O157:H7 in vegetables as 10.3%, 17.5%, 18.2% and 21.66% respectively.

Despite low prevalence of *Escherichia coli* O157:H7 recorded in the present study, vegetables cultivated in Samaru, Zaria may be considered of public health concern with regards to their microbiological quality. The isolation of this pathogen in vegetables poses food safety problem. *E. coli* O157:H7 with its abundance contaminated soil, do not have penetrating power into spinach, uptake and internalization was found to occur in spinach leaves after contaminated water was dropped on the leaves (Benard, 2008). The pathogen also, adheres strongly to tomato skin, spinach leaves and roots of alfalfa sprouts. Adhesions to these surfaces are mediated by pili (Jeter and Matthysse, 2005). Expression of curli on the surface of non-pathogenic *E. coli* was shown to be sufficient to enable bacterial attachment to Alfalfa roots, but deletion of pili genes in *E. coli* O157:H7 did not abolish adhesion, suggesting that other attachment factors were involved (Jeter and Matthysse, 2005). The tomatoes might be contaminated either in the field or in the market place when spread on the bench or where the tomatoes might be washed before sell with contaminated water (Jeter and Matthysse, 2005). The current study reveals the anti-bacterial sensitivity effect of salt and vinegar against *Escherichia coli* O157:H7.

The effect shifts from static/inhibitory (salt 2.5mg/ml and 12.5%v/v) to cidal (salt 10mg/ml and vinegar 25% v/v). Salt and vinegar are effective against *E. coli* O157:H7 (Etani *et al*., 1998). Salt affects the cell of the *E. coli* O157:H7 through high osmotic pressure with resultant severe cell wall damage (Hajmeer *et al*., 2006). The cell damage increases with increase in salt concentration (Hajmeer *et al*., 2006). Vinegar is known to contain acetic acid, which affects the bacterial plasma membrane with resultant cidal effect on the bacterial cell (Etani *et al.*, 1997).

CONCLUSION

Leafy vegetables are probable vehicles for transmitting *E. coli* O157:H7.

Disinfection of vegetables with salt and vinegar at different concentrations could be a promising preventive measure of microbial contamination of vegetables.

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