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ISOLATION AND IDENTIFICATION OF *ESCHERICHIA COLI* FROM RAW VEGETABLES IN SELECTED VEGETABLE MARKETS OF SOKOTO METROPOLIS

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ABSTRACT
Bacterial contamination of vegetables is a major source through which animals and humans get infected with diseases. *Escherichia coli* is commonly found in the digestive tracts of humans and animals and is considered as part of the normal bacteria of the intestine. *E. coli* are a large and diverse group of bacteria, the organism helps to suppress growth of harmful bacteria and aids in the synthesis of vitamins but some can affect animals as well as humans. The study was aimed at isolation and identification of *E. coli* from raw vegetables in selected vegetable markets in Sokoto metropolis. Samples of cabbage, lettuce, spinach, pumpkin and water leaves were obtained from four (4) different markets. Isolation and identification of *E. coli* was carried out using McConkey agar and eosin methylene blue agar. Confirmation was done using biochemical tests. The result obtained showed a prevalent of 20 % (4). This indicates that vegetables could be an important potential source of transmission of *E. coli* to both animals and humans.

KEYWORDS: *Escherichia coli*, Vegetables, Sokoto and Bacterial contamination.

INTRODUCTION
In 1885, a German bacteriologist, Theodore Von Escherichdiscovered *Escherichia coli* bacterium. It is commonly found in the digestive tracts of humans and animals and is considered as part of the normal bacteria of the intestine (Todar, 2005). *E. coli* are a large and diverse group of bacteria. Many of the *E. coli* strains are harmless. The organism helps to suppress growth of harmful bacteria and aids in the synthesis of vitamins but some can affect animal as well as human health. The very first outbreak of *E. coli* O157:H7 happened during 1993 in America at the box restaurant which led to the death of four children and seven hundred people were infected with severe stomach cramp and bloody diarrhea. It had been known that the customers were served with undercooked hamburgers and during that time it was known as the hamburger disease (Giusti *et al*., 2003). *E. coli* is normally detected in food, water and clinical specimens. It is reported that *E. coli* is most often found in meat especially *E. coli* O157:H7. It is also found in fruits and raw vegetables during foodborne outbreaks for example lettuce (Ackers *et al*., 1998), alfalfa sprouts (Breuer *et al*., 2001) and cantaloupe (Del Rosario & Beuchat, 1995). Other food products including raw milk, yogurt, apple juice and apple cider which had contact with cattle are also reported as sources of transmission. *E. coli* lives in the stomach of cattle for weeks to months, therefore the probability that the organism will survive is high (Massett, 2001).

The source of infection in animals are mostly through eating raw vegetables especially spinach, lettuce, cabbage, waterleaf and fluted pumpkin leaf(ugwu leaf). Animals like goats, sheep, rabbits, and pigs eat these vegetables as their normal food. Therefore, vegetables containing the virulent strain of *E. coli* will have chances of causing the earlier mentioned disease to the group of animals. Sources of infection to human beings of this virulent organism could be of animal origin and food, including the aforementioned vegetables (Willshaw *et al*., 2001). Reservoirs of *E. coli* in animals include sheep, goats, pigs, horses, dogs, cats, deer, and domestic and wild avian species inclusive (Fisher *et al*., 2001, Bruntu *et al*., 1993, Wallaci *et al*., 1997). Cattle are considered the principal reservoirs of this pathogen; sheep and goats have similar role to that of cattle in terms of human infection (Kudua *et al*., 1977; Hevelenk *et al*., 1998; Barkocy-Gallagher *et al*., 2001). *E. coli* can be transmitted via direct access that results in vehicle transmission to both animals and human beings (Trevena

et al., 1999). Infection can also be water-borne (Ackman *et al*., 1997, Rice *et al*., 1999, Olsen *et al*., 2002). There have been reports of infection across the world due to contamination of vegetables with pathogens while infield, improperly composed manure, contaminated water and poor hygienic practices of farm workers and selling places (WHO, 1994).

According to the Centers for Disease Control and Prevention (CDC), the recent outbreak that happened in vegetables is the multi-state outbreak of *E. coli* O157:H7 infections from fresh spinach. This outbreak occurred in 26 states of North America infecting 199 people and killing 3 people (CDC, 2006). For the recent years, *E. coli* O157:H7 appeared to be the popular pathogenic strain causing food-borne outbreaks and illness. Multistate outbreak of *E. coli* O157:H7 infections associated with the consumption of pre-packaged cookie dough in United States had infected 23 people (CDC, 2009). It is reported that the patients had eaten refrigerated pre-packaged Nestle Toll House cookie dough products raw, the most recent *E. coli* O157:H7 food-borne outbreak is associated with beef from Fairbank Farms causing multistate outbreak in United States. Most ill persons from this outbreak had consumed ground beef with several purchasing the similar product from a common retail chain (CDC, 2009).

In Malaysia, 'ulam' is a name given to a group of vegetables that are eaten raw and is also known as salad. A variety of vegetables are used to make 'ulam', the more common ones are spinach, yard-long beans and Indian pennywort (Chai *et al*., 2007). Due to the facts that 'ulam' is eaten raw, there is possibility that the naturally contaminated vegetables could be an important vector in the dissemination of *E. coli* O157:H7 to other surfaces in the domestic kitchens.

It is learned that kitchen handling practice is also important for protecting our daily eaten foods. Cross-contamination of bacterial and viral pathogens in domestic kitchens is thought to be a major contributing factor for epidemic foodborne illnesses after an outbreak in a school canteen in Japan in 1996 which is associated with *E. coli* (Bolton, 2006). During preparation of food in domestic kitchens, bacterial and viral pathogens can be transferred from many places such as cutting boards and through hands to other food sources (Montville, 2001). This study is aimed at isolation and identification of *E. coli* from raw vegetables in selected vegetable markets in Sokoto metropolis.

MATERIALS AND METHODS

Study Design

The study was conducted and concluded within a period of 8 weeks (9th of April to 9th of June 2018). Samples of cabbage, lettuce, spinach, pumpkin and water leaves were obtained from four (4) different markets (i.e. total of 20 samples). The study is cross-sectional.

Sample Collection

Sterile gloves were used to sample 10 g each of cabbage, lettuce, spinach, waterleaf, and pumpkin leaf in the morning and afternoon from four different markets within Sokoto metropolis and were transported to the laboratory in new polythene bags.

Isolation and Identification of *E. coli*

The isolation media used in this research work were nutrient broth, McConkey agar, Eosin Methylene Blue agar (EMBA) and nutrient agar slant was used for storage of the suspected colonies.

Sample Processing

A millimeter of fluid from each vegetable sample washed with sterile water was withdrawn, inoculated into 9mls of peptone water in separate test tubes (for each sample) and incubated at 37°C for 24hours. A loop of the mixture (sample and peptone water) was inoculated onto McConkey Agar and incubated at 37°C for 24hours. A typical pinkish colony indicating lactose fermenter was picked up using sterile loop, and sub-cultured onto Eosin Methylene Blue Agar (EMBA) and incubated at 37°C for 24hours. *E. coli* colonies showing green metallic sheen with black center were picked and streaked in nutrient agar slants, then incubated at 37°C for 24hourrs and preserved for further study.

Gram Staining

An inoculating loop was used to pick the inoculum and a smear was made on a clean, grease-free glass slide and heat-fixed and the smear was flooded with crystal violet and left to stand for 1 minute. The slide was tilted slightly and gently rinsed with distilled water from a wash bottle and the smear was then flooded with Gram's iodine and left to stand for 1 minute. The slide was tilted slightly and rinsed with distilled water from a wash bottle gently. The smear appeared as a purple circle on the slide; the slide was tilted slightly and 95% ethyl alcohol was applied drop by drop for 5-10 seconds until the alcohol runs almost clear. Care was taken not to over-decolorize, it was immediately rinsed with water, and gently flooded with safranin to counter-stain and let stand for 45 seconds. The slide was tilted slightly and gently rinsed with distilled water from a wash bottle and the slide was shaken to remove most of the water and air-dried. The smear was then viewed using a light microscope under oil-immersion. Pinkish colouration, short-rods were observed using microscope at x100 (oil immersion) magnification which were considered presumptive *E. coli* (Wilson & Miles, 1995).

Colony Counting

Pour plating technique was used. 1ml of the serial dilution was first added to a sterile petri dish followed by the addition of the molten media. Both were mixed gently, covered and left for 1hour to solidify. It was then turned upside down and incubated at 37°C for 24hours. The growths were enumerated using a colony counter

and the counts expressed in colony forming units per ml (CFU/ml).

Biochemical Tests

Indole Test

Peptone water was prepared, and 4mls put in a test tube and inoculated. It was incubated at 37°C for 24-28 hours and 0.5mls of Kovac's reagent was added to the culture, and observed for the presence of red ring or layer at the top of the tube which indicates a positive result.

Methyl Red (MR) Test

Methyl red –Voges Proskauer (MR/VP) broth was prepared, put in a test tube and inoculated. It was incubated at 35°C for up to 4 days and 5 drops of the methyl red indicator solution was added. A positive reaction is indicated by change in color of the medium to red within a few minutes.

Voges-Proskauer (VP) Test

Methyl red –Voges Proskauer (MR/VP) broth was prepared, put in a test tube and inoculated. It was incubated at 35°C for 24 hours and Darrit's reagent indicator solution (consisting of alpha naphthol and KOH) was added. The tube was shaken gently to expose the medium to atmospheric oxygen and left undisturbed for 10-15 minutes. A positive reaction is indicated, by colour change of the medium to red 15 minutes or more (but less than an hour) after the addition of the reagents.

Citrate Utilization Test

Simmons citrate agar was prepared and put in a slant bottle. The sample was then inoculated by touching the tip of a needle to a colony and putting in the Simmons citrate agar. It was then incubated at 35°C-37°C for 18-24 hours, absence of colour change from deep forest green to Prussian blue is an indication of citrate negative organism typical of *Escherichia coli*.

Calculation of Prevalence

The prevalence was calculated using the following formula:

$$\text{Total Prevalence (\%)} = \frac{\text{TOTAL NUMBER OF } E.COLI \text{ POSITIVES}}{\text{TOTAL NUMBER OF SAMPLES}} \times 10($$

Prevalence of *E. coli* in each vegetable type

$$= \frac{\text{NUMBER OF } E.COLI \text{ POSITIVES ON THE VEGETABLE}}{\text{TOTAL NUMBER OF SAMPLES}} \times 10($$

RESULTS

A total of 20 samples, 4 samples each of cabbage, lettuce, spinach, pumpkin (ugwu) leaf and water leaf from 4 different markets were obtained and screened for the presence of *E. coli*. Eighteen of the samples (90%) were found to be lactose fermenters (4 was from cabbage, 3 from lettuce, 4 from spinach, 3 from pumpkin leaf and 4 from water leaf). 4 samples out of the lactose fermenters {22.2%} were positive for *E. coli* when cultured on Eosin Methylene Blue Agar of which 1 was from cabbage, 2 from spinach and 1 from waterleaf. After conducting IMViC biochemical test, all the four samples were onfirmed and identified to be *E. coli*. The specific prevalence of *E. coli* in cabbage, lettuce, spinach, pumpkin leaf and waterleaf is 5%, 0%, 10%, 0% and 5% respectively, while the overall prevalence is 20%. The result of the colony counts also indicates that the vegetables were highly contaminated especially the ones from Sokoto old Market.

Table 1: Isolation and biochemical identification of bacteria from raw vegetables sold in some Sokoto markets.

S/NO	SAMPLE	COLONY COUNT (CFU/ml)	MC CONKEY	E.M.B	GRAM REACTION	IND OLE	MR	VP	CITRATE	ORGANISM IDENTIFIED
1.	A1	15×10^4	+	-						
2.	B1	10×10^4	-	-						
3.	C1	12×10^4	+	-						
4.	D1	14×10^4	+	-						
5.	E1	12×10^4	+	+	- short rods	+	+	-	-	*E. coli*
6.	A2	31×10^4	+	+	- short rods	+	+	-	-	*E. coli*
7.	B2	TNTC	+	-						
8.	C2	25×10^4	+	+	- short rods	+	+	-	-	*E. coli*
9.	D2	22×10^4	+	-						
10.	E2	TNTC	+	-						
11.	A3	11×10^4	+	-						
12.	B3	13×10^4	+	-						
13.	C3	15×10^4	+	+	- short rods	+	+	-	-	*E. coli*
14.	D3	13×10^4	-	-						
15.	E3	17×10^4	+	-						
16.	A4	12×10^4	+	-						
17.	B4	13×10^4	+	-						
18.	C4	9×10^4	+	-						
19.	D4	14×10^4	+	-						
20.	E4	12×10^4	+	-						

Sample Column: A= Cabbage, B= Lettuce, C= Spinach, D= Pumpkin leaf, E= Water leaf.
1= New Market, 2= Old Market, 3= Mabera Market, 4= UDUS Market.

Prevalence of *E. coli*

The total prevalence was 20 %. The Prevalence of *E. coli* in each vegetable type is presented in Table 2.

Table 2: Prevalence of E. coli on Vegetable Types.

S/No.	Vegetable Type	% Prevalence
1	Cabbage	20.0
2	Lettuce(salad)	0.0
3	Spinach	10.0
4	Waterleaf	0.0
5	Pumpkin	5.0

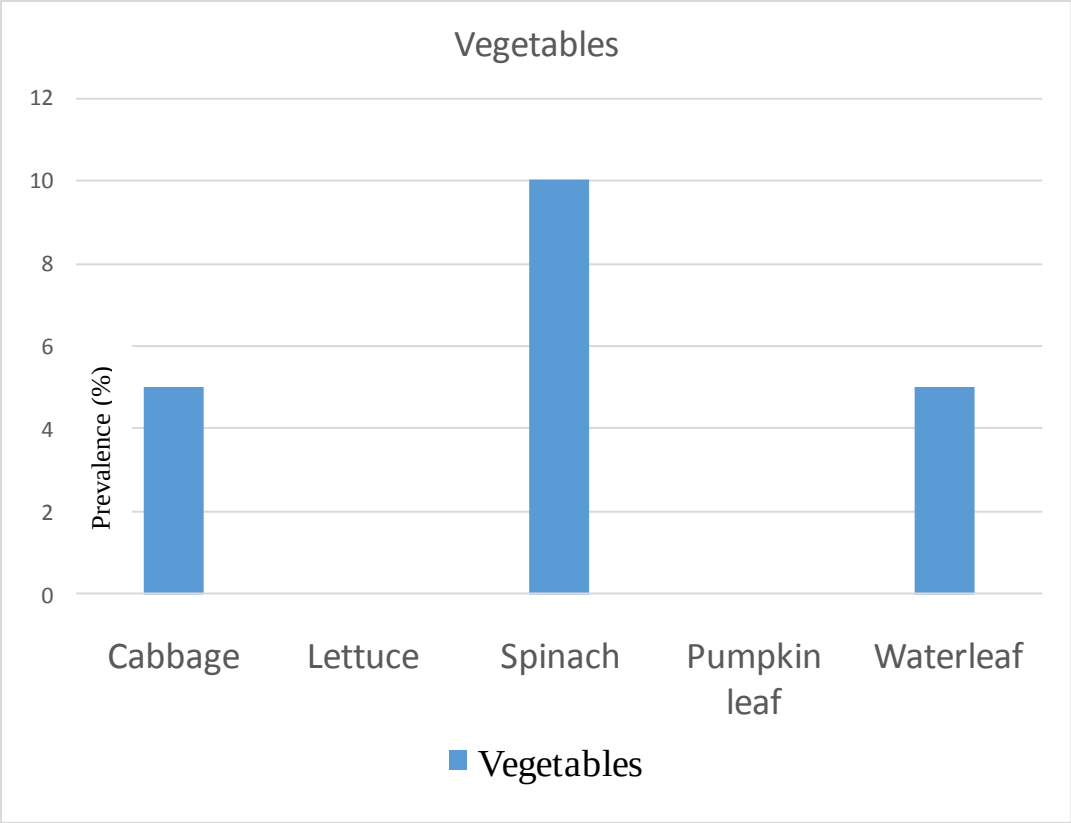

Figure 1: Prevalence of *E. coli* in the varieties of vegetables.

DISCUSSION

In this study, a total of 20 samples were collected, out of which 4 (20.0%) were positive for *E. coli*. *E. coli* are strongly associated with Haemolytic Uraemic Syndrome (HUS), leading to acute renal failure in children and Haemorrhagic Colitis (HC) in adults and also associated with diarrhea in animals. Studies also show that environmental pollution by human waste is a potential source of soil and water contaminations with *E. coli* and therefore an important source of a transmission by vegetables that are cultivated on soil and river water that are used for washing them.

A similar research carried out in the Kurdistan region by Ali, Y.S. *et al.,* (2013) of the Department of Biology, Faculty of Science, University of Duhok, Iraq yielded a prevalence of 19.5% after isolating *E. coli* from 39 out of 200 samples of vegetables.

Another research made by Mariam Bako (2014) of the Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria yielded a prevalence of 63.6% after identifying *E. coli* in 19 out of the 30 vegetable samples obtained.

In another similar work done by Gonzalez, J. *et al,* (2017) in Argentina, *E. coli* was detected in 38.6% of the samples. 144 samples were positive for *E. coli* out of the 373 samples.

Study of *E. coli* presence in raw vegetables by Mritunjay S.K. (2017) yielded 16.7% prevalence after detecting *E.*

coli in 80 out of the 480 samples collected. The research was carried out in Jharkhand, India.

These differences in the results could be attributed to many factors such as sample size, methodology used for isolation and identification, geographical variations, seasonal variation, agricultural methods for vegetable production and hygienic precautions.

In Sokoto Nigeria, the likelihood of contamination of vegetables with this organism increases with increased use of human manure for vegetable production and improper washing of vegetables purchased from various markets in the metropolis. Many varieties of these vegetables are cultivated around Sokoto metropolis in both wet and dry seasons (MANR, 2005). Sources of contamination could be from soil contaminated by faeces (especially when used as manure), water or by the vegetable handlers (Smith, 1998). During the dry season, irrigation using water from ponds or wells; and during the rainy season, rain water running on the ground surface washing away faeces which leads to contamination of these vegetables.

CONCLUSION

The study has clearly established the presence of *E. coli* in vegetables especially cabbage, spinach and water leaf sold and consumed in Sokoto metropolis and most likely sources of contaminations are animal wastes and human faeces which are used as manure.

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