Isolation and Identification of *Escherichia coli* O157:H7 from Raw Salad Vegetables Sold in Sokoto Metropolis, Nigeria

Bahira, B. Y[3]., Muhammad, U-K[1]., Baki, A. S[1]. and Gambo, S.[2]

1. Department of Microbiology, Usmanu Nigeria Danfodiyo University, Sokoto,
2. Department of Microbiology Federal University Gusau, Nigeria
3. State College of Basic and Remedial Studies, Sokoto

Corresponding Author: garkuwacrc@gmail.com/08133748800

Abstract: The presence of *Escherichia coli* O157:H7 in fresh salad vegetables was evaluated in this study. One hundred and fifty (150) Salad vegetable samples (lettuce, cabbage and spring onion) were aseptically collected from two different markets (Ramin Kura and Meat and vegetables market) in Sokoto metropolis. Isolates from the samples were fully characterized by using standard methods. Antimicrobial susceptibility patterns of seven (7) E. coli O157H7 isolates were investigated using 8 commonly used antibiotic, showed level of antimicrobial resistance. Out of the 19 *E. coli* identified only 7 were *E. coli* O157:H7. The occurrence of *E. coli* O157:H7 in lettuce, cabbage and spring onion were 2 (1.33%), 3(2.00%) and 2(1.33%) respectively. The highest occurrence rate observed from this study was in cabbage 3(2.00%). Samples obtained from Meat and vegetables market have more occurrence of *E. coli* O157:H7 than samples obtained from the Ramin kura market. Oflaxacin 40(mm), ciprofloxacin 33 (mm) and nitrofurantoin 32 (mm) showed high antibacterial activity while Augmentin did not show any activity. Presence of *Escherichia coli* O157:H7 in salad vegetables serve as potential risk of infection to the consumers.

Keywords: Antibiotic susceptibility, contamination, *Escherichia coli* O157:H7 and fresh salad vegetables

INTRODUCTION

E. coli O157:H7 was first recognized as a cause of human gastrointestinal disease in 1982 and was traced to contaminated hamburger meat, during an outbreak investigation of acute painful bloody diarrhea known as hemorrhagic colitis (Rangel *et al*., 2005). Enterohemorrhagic *Escherichia coli* O157:H7 is the major cause of foodborne and waterborne illnesses that cause bloody diarrhea, hemorrhagic colitis (HC), and life-threatening hemolyticuremic syndrome (HUS) worldwide (Kendal *et al*., 2012). Infections with *E. coli* O157:H7 are the most common cause of acute renal failure in infants and young children (Gerber *et al*., 2002).

Vegetables are exceptional dietary source of nutrients, micronutrients, vitamins and fibre for human beings hence vital for health and fitness. Foods rich with fresh vegetables are especially valuable for their ability to prevent deficiencies of vitamin C and vitamin A and also reduce the risk of several diseases (Kalia and Gupta, 2006).

Tambekar *et al*. (2006) reported that bacterial contamination of salad vegetables was linked to the fact that they are usually consumed without any heat treatment. These vegetables can become contaminated with pathogenic microorganisms during harvesting, through human handling, harvesting equipment's, transport containers, wild and domestic animals. Pathogens from the human and animal reservoir as well as other environmental pathogens can be found at the time of consumption. In other studies the pathogenic microorganisms (especially bacteria) originating from intestinal tracts of animals or humans are more likely to contaminate vegetables through faeces, sewage, untreated irrigation water or surface water (Harris *et al*., 2003). There is always a chance of *E. coli* O157:H7 being present in vegetables grown in manure-fertilized soils (Islam *et al.,* 2004). The potentially high risk associated with *E. coli* O157:H7 in fresh vegetables, make its presence in any food materials a serious public health concern (Hussein, 2007). This present study was undertaken to isolate, identify and to determine antibiotic susceptibility to commonly used antibiotics, of *Escherichia coli* O157:H7 from salad vegetables commonly consumed in Sokoto metropolis.

MATERIALS AND METHODS

Collection and Processing of Vegetable Samples

Hundred and fifty (150) samples of commonly-consumed fresh salad vegetables (lettuce, cabbage and spring onion) were aseptically collected during the early morning hours in two different markets (Ramin Kura and Meat and Vegetable Market) all in Sokoto metropolis. Five (5) samples of each salad vegetables (lettuce, cabbage and spring onion) were collected from the two different markets each day for five days. The Samples were collected in new sterile polythene bags, labeled and transported, to the laboratory in the Department of Microbiology, Usmanu Danfodiyo University, Sokoto, for immediate analysis (APHA, 1998).

Isolation and identification of *E. coli* isolates

A 25g of each vegetable sample was weighed, rinsed in a beaker containing 100 mL of sterile physiological saline. One milliliter (1ml) of each of the washing was added to a test tube containing 9ml of sterile physiological saline (0.85% NaCl). Serial dilution was carried out from 10^{-1} to 10^{-5} tubes and 0.1 ml of 10^{-5} each was plated on Eosin methylene blue (EMB) agar. They were then incubated at 37°C for 24h. Bluish black colonies with metallic sheen (typical of *E. coli*) were streaked on to Nutrient agar plate for bacterial colony purification (Odu and Okomuda, 2013). After the purification, the isolates were maintained on nutrient agar slant, and kept in refrigerator at 4^{o}C for further analysis.

Selective Plating and Identification of *E. coli* O157:H7Colonies

Pure cultures of all positive *E. coli* growing on (EMB agar slants) were streaked onto Telluritte-Cefixime Sorbitol MacConkey agar (CT-SMA) plates and incubated at 37□C for 24 hours. A suspected *E. coli* O157:H7 appearing as non-sorbitol fermenter colony (NSFC) was characterized as having a slightly transparent, almost colorless with a weak pale brownish appearance. Presumptive colonies were Gram stained for the presence of Gram-negative rods (Prescott *et al*., 2005). Individual discrete colonies from the CT-SMAC agar plates were picked and sub-cultured onto nutrient agar slants and incubated at 37^{o}C for 24 hours. The slants were then refrigerated at 4^{o}C for further biochemical analysis (Voges-Proskauer reactions, indole, citrate, urea hydrolysis, triple sugar iron test (TSI), and H_2S production) (Reuben and Makut, 2014).

Serological Test

E. coli O157:H7 positive colonies were serologically confirmed by using *E. coli* O157:H7 latex agglutinations assay, containing latex particles coated with antibodies specific for *E. coli* O157 and *E. coli* H7 antigen. Identification of *E. coli* O157:H7 was carried out following the manufacturer's instructions, hence, colonies that agglutinated to the separate antisera were considered to be *E. coli* O157:H7 (Reuben and Makut, 2014).

Antibiotic Susceptibility Testing

The susceptibility of the *E. coli* O157:H7 isolates against antimicrobials was determined by Kirby-Bauer disc diffusion method on Mueller-Hinton agar. The inoculum was prepared at a density adjusted to a 0.5 McFarland turbidity standard solution. Bacterial suspension was inoculated on Mueller Hinton Agar (MHA) medium using sterile swab stick. Plates were dried for 15 minutes and 8 commercially available antibiotic discs were applied on the plate surface and allowed to stand for 30 minutes to allow the antibiotics to diffuse in the agar medium. The antibiotic discs used were: Cefixime (5µg), Cefuroxime/Sodium (30µ), Ciprofloxacin (5µg), Gentamycin (10µg), Nitrofurantoin (300µg), Ofloxacin (5µg), Augmentin (30µ), and Ceftizidime (30µ). Each plate was incubated in an inverted position overnight at 37 °C. Sensitivity was recorded after 24 hours of incubation by measuring the zone of inhibition formed around the antimicrobial discs. Results were expressed as Sensitive, Intermediate and Resistant (CLSI, 2012).

RESULTS AND DISCUSSION

Biochemical characteristic of the isolates showed them to ferment sucrose, lactose and glucose respectively. The isolates were Indole and Methyl red positive but Voges-Proskaure and Citrate negative (Table 1).

This study revealed that out of 19 (12.66%) *E. coli* isolated, only 7 (4.66%) were *E. coli* O157:H7. The occurrence of *E. coli* O157:H7 in lettuce, cabbage and spring onion were 2 (1.33%), 3(2.00%) and 2(1.33%) respectively. The highest occurrence rate observed from this study was in cabbage 3(2.00%) (Tables 2 and 3). Samples obtained from Meat and vegetables market have more occurrence than samples obtained from the Ramin Kura market. The occurrence of *E. coli* O157:H7 in the vegetables observed in this study is very much in agreement with previous studies in other parts of the world by Mora *et al.* (2000) 18.2% in Spain, Benard, (2008) 21.66% South Africa, Enabulele and Uraih (2009) 19.5% and Reuben and Makut, (2014) who reported the occurrence rates of 7.5% in Nigeria. Despite the low occurrence of *E. coli* O157:H7 in vegetables as recorded from this study, pre- and post-harvest handling of vegetables in Sokoto metropolis and Nigeria in general is a great cause of concern regarding their microbiological quality. Due to the fact that infectious dose of *E. coli* O15:H7 is very low, it can cause infections even at unnoticeable levels (Eric *et al*., 2008). The presence of *E. coli* O157:H7 indicates contamination by faecal matter and possible presence of other enteric pathogens known to be causative agents of food borne gastroenteritis and other bacterial disease (Adebayo-Tayo *et al*., 2012). The isolation of this pathogen in vegetables therefore poses food safety problem.

It has been observed that most of the vegetables in Nigeria are grown with irrigated water that is in contact with grazing cattle and this can be a source of contamination (Solomon *et al.,* 2012). Another likely source of contamination with these organisms is the vehicle on which the vegetables are transported to the various sale points. It has also been observed that vegetables are transported using the same vehicles used in transporting animals from the farm to the sell point with the dung as likely to be the source of contamination. Although studies have shown that *E. coli* O157:H7 could contaminate vegetables, especially through irrigation water and other sources such as soil (Solomon *et al.,* 2012). These results obtained were similar with the results of Sharif and Arafa (2004), who found *E. coli* O157:H7 in 11.7% of studied vegetables, and Abong (2008), who found prevalence of *E. coli* O157:H7 to range from 0% to 33% in onions and cabbage respectively. However, the present study disagreed with the results recorded by Enabulele and Uraih (2009) who did not detect *E. coli* O157:H7 among all the isolated *E. coli* from the vegetables analyzed. The antimicrobial susceptibility patterns of 7 *E. coli* O157:H7 isolated from vegetable samples (lettuce, cabbage and spring onion) using 8 commonly used antibiotics showed some level of antimicrobial resistance in this study. *E. coli* O157:H7 isolates were sensitive to oflaxacin, ciprofloxacin, gentamycin and nitrofurantoin but were resistant to augmentin, ceftizidine, cefixime and cefuroxime (Table 4).

Table 1: Biochemical characteristic of the isolates before confirmation to be *E. coli*

S/N	Test	Result
1.	Sucrose	+
2.	Glucose	+
3.	Lactose	+
4.	H_2S production	+
5.	Voges-Proskaure	-
6.	Indole	+
7.	Citrate	-
8.	Methyl red	+

KEY: - = negative, **+ =** positive

Table 2: Serological characteristics of *E. coli* O157:H7 among the *E. coli* isolates obtained from the vegetables samples

S/N	Isolates	Reactions
1	EC_1	-
2	EC_2	-
3	EC_3	+
4	EC_4	-
5	EC_5	+
6	EC_6	+
7	EC_7	+
8	EC_8	-
9	EC_9	+
10	EC_{10}	-
11	EC_{11}	-
12	EC_{12}	-
13	EC_{13}	+
14	EC_{14}	-
15	EC_{15}	+
16	EC_{16}	-
17	EC_{17}	-
18	EC_{18}	-
19	EC_{19}	-

Key: EC= *Escherichia coli*, + = positive, - = Negative

Table 3: Occurrence of *E. coli* O157 in the raw vegetables.

S/N	Samples	No of samples	No of *E. coli* isolated	No of confirmed *E. coli* O157:H7
1	Lettuce	50	8 (5.33%)	2 (1.33%)
2	Cabbage	50	4 (2.66%)	3 (2.00%)
3	S/onion	50	7 (4.66%)	2 (1.33%)
		Total = 150	19(12.66%)	7(4.66%)

Table 4: Antibiotic sensitivity profile of *E. coli* O157:H7 isolates obtained from the vegetable samples.

		ANTIBIOTICS							
S/N	ISOLATES	CAZ (30µ)	CRX (30µ)	GEN (10µg)	CXM (5µg)	OFL (5µg)	AUG (30µ)	NIT (300µg)	CPR (5µg)
1	EC_3	-	-	16	-	-	-	30	-
2	EC_5	08	09	24	-	40	-	32	20
3	EC_6	-	-	14	10	21	-	15	19
4	EC_7	-	-	13	15	17	-	27	33
5	EC_9	-	-	18	-	-	-	18	-
6	EC_{13}	-	-	18	11	32	-	27	31
7	EC_{15}	-	-	12	-	-	-	15	-

KEY: EC = *Escherichia coli* O157:H7, CAZ = Ceftizidine, CRX = Cefuroxime, GEN = Gentamicin, CXM = Cefixime, OFL =Ofloxacin, AUG =Augmentin, NIT = Nitrofurantoin, CPR = Ciprofloxacin

The presence of *E. coli* is of public health significance as *E. coli* has been reported to be important in horizontal transfer of resistance among other pathogenic bacteria (Todd and Dundas, 2001). Johnson *et al.* (2007) stated that one suspected source of drug-resistant *E. coli* in humans is the use of antimicrobial drugs in agriculture. As humans are continuously exposed to bacteria in the environment, the accumulation of resistance genes in soil due to the spreading of manure is likely to contribute to the threat of antimicrobial resistance in the therapy of infectious diseases (Heuer *et al.,* 2011). Udo *et al.* (1994) also reported multiple antibiotic resistance in *E. coli* obtained from ready to eat vegetables in Calabar, Nigeria. Acquired resistance to first line antimicrobial agents may complicate the management of infections caused by *E. coli* O157:H7. Hence, the multidrug resistance exhibited by *E. coli* 0157:H7 is of major public health concern especially in Nigeria and other developing countries.

CONCLUSION AND RECOMMENDATION

The isolation of *E. coli* O157:H7 from the salad vegetables has been reported to represent an imperative risk to public health globally. Thus, the present study adds new insight to the existing knowledge on the microbiology of the commonly consumed raw salad vegetables in Sokoto, Nigeria as follows: The results of these study revealed that vegetable salads may contain pathogenic bacteria; food pathogens can survive in vegetable salads, and thereby represent a risk to the consumers in regard to food borne disease. Bacterial contamination may be present due to improper handling, unhygienic transportation condition and improper storage. Other sources could be the result of post-harvesting processing and unhygienic distribution. It was observed that the *E. coli* O157:H7 isolates were sensitive to oflaxacin, ciprofloxacin, gentamycin and nitrofurantoin but were resistant to augmentin, ceftizidine, cefixime and cefuroxime. Hence the hygiene practice must be improved in all the vegetables supply chain to avoid risk of contracting diseases.

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