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Research paper

Shiga-toxin producing *Escherichia coli* (STEC) and other enterobacteriaceae associated with ready-to-eat salad

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Abstract

Ready-to-eat (RTE) salads sold in Nigeria are poorly delineated sources of human exposure to pathogenic microorganisms. In this study, we investigated the current situation in Benin City, Edo state, Nigeria. Twenty-four samples of RTE salad were obtained from different open markets, and the presence of Shiga toxin-producing *Escherichia coli* (STEC) and other enterobacteriaceae were determined by established methods using both selective and chromogenic agars. All RTE salad samples were found to habour *Escherichia coli* while 16.7% were further confirmed for the presence of STEC. Other Enterobacteriaceae present included *Klebsiella* spp, *Proteus* spp, *Enterobacter* spp, *Serratia* spp and *Salmonella* spp. The antibiogramic profile revealed that all bacterial isolates obtained were resistant to augmentin and amoxicillin while only 11.1% were resistant to ciprofloxacin and ofloxacin. The percentage resistance for the Shiga-toxin producing strains of *E. coli* was 60% while *Serratia* showed resistance to all the antibiotics used. The results of this study showed that RTE salad sold in Benin City, Edo State, Nigeria could be a source of public health concern, and effort should be made to avert possible outbreak.

Keywords: *Salad; Outbreak; Shiga Toxin-Producing E. coli; Enterobacteriaceae; Public Health.*

1. Introduction

Ready-to-eat (RTE) foods are food products that do not require further processing to ensure safety and palatability prior to consumption. Examples of RTE food include lunch-on meats, salad, bakery products and cheeses. Salads are made with fresh vegetables such as lettuce, spinach, carrot, cabbage, cucumber, and sprout and are known as one of the important sources of vitamins, nutrients and fiber (Grant, 2008). The demand for fresh vegetables in Nigeria is on the increase as people are now more concerned with healthy lifestyle and balanced diet (Warriner et al., 2009; Olaimat and Holley, 2012). In addition to the beneficial health effect of salad, they could be sources of outbreak of several infections and illnesses. Also, the outbreaks of food infections associated with RTE salad are said to be on the increase (Oluwatosin and Elna, 2012). In September 2006, pre-packaged fresh salad were recalled by the Food and Drug Administration (FDA) in the United States of America (USA) as a result of an *Escherichia coli* outbreak in California, USA (Oluwatosin and Elna, 2012). Also in the same month there was an *E. coli* 0157: H7 outbreak linked to lettuce from Taco Bell restaurants in Northern USA (Oluwatosin and Elna, 2012). Similarly, in 2011, Germany reported a nationwide outbreak of haemolytic uremic syndrome (HUS) caused by Shiga toxin-producing *E. coli* (STEC). This outbreak was associated with the consumption of fresh salad vegetables which resulted in 54 deaths (Frank et al., 2011). The Public Health Agency of Canada also reported in June 2011, one suspected case of *E. coli* O104 infection (without HUS), with travel history to Northern Germany and with link to a confirmed case of *E. coli* O104 infection in Germany.

Additionally, the occurrence of microorganisms especially the enterobacteriaceae in RTE salad is an indication of the sanitary conditions of the processing process as well as the microbiological condition of raw product at the time of processing (Nguyen and Carlin, 1994). Other factors that could influence the proliferation of microorganisms in RTE salad include the hygiene of the personnel involved in the preparation, the utensils, equipment, storage pattern and the processing environment. These factors may lead to a rise in the number of pathogens in salad at infectious doses.

STEC is associated with haemorrhagic colitis (HC), haemolytic-uremic syndrome (HUS) and renal failure in children (Coombes et al., 2008). Although most studies have shown that outbreaks of HC and HUS are associated with *E. coli* O157:H7 (Pradel et al., 2001; 2008), some studies have also reported high prevalence of non-O157 and O157: H-(non-motile) serotypes in human diseases and from animals and related food products (Coombes et al., 2008; Pradel et al., 2001)

Many types of food have been implicated in STEC outbreaks: undercooked bovine meat products (minced meat, hamburgers), fresh produce such as lettuce, bean sprouts and spinach, unpasteurized apple cider, and raw milk. STEC outbreaks have also been waterborne, owing to faecally contaminated drinking-water or contaminated water during swimming (WHO, 2011).

Considering the potential public health impact of these isolates (STEC and the Enterobacteriaceae), this study was aimed at isolating Shiga-toxin producing strains of *Escherichia coli* and other Enterobacteriaceae of public health concern from RTE salad sold in Benin City, Edo State, Nigeria, and to determine their antimicrobial sensitivity pattern.

2. Materials and methods

2.1. Materials

The Nutrient agar, Eosin methyl blue (EMB) agar, Muller Hinton agar and MacConkey agar used in this study were obtained from Titan Biotech Ltd (Rajasthan, India), while the CHROMagar STEC base was obtained from CHROMagar (Paris, France).

2.2. Sample collection

Twenty-four samples of ready-to-eat salad were obtained from two major markets (Oba and Eki-Osa) in Benin City, Edo State, Nigeria. All samples were obtained between January and February 2016.

2.3. Microbiological analysis

The mean coliform and mean enterobacteriaceae counts were determined as previously reported (Omoruyi et al., 2011). Briefly, 25g of salad samples were placed in 225ml of peptone water, following which serial dilutions were made. One milliliter each of the serial dilutions (4^{th} and 5^{th}) was transferred to already prepared Eosin EMB, MacConkey agar and Nutrient agar plates. The respective agar plates were then incubated in triplicates at 37^0C for 24hours.

2.4. Identification of bacterial isolates

Clearly distinct colonies were identified based on their cultural, morphological and biochemical characteristics, principally characteristic of the Enterobacteriaceae family. Pure cultures of all colonies exhibiting typically dark colonies with green metallic sheen on EMB agar and pink colonies on MacConkey agar were subcultured and re-plated on CHROMagar STEC base. *E. coli* strains habouring the shiga-toxin gene were noted for the mauve colouration (Figure 1) while other enterobacteriaceae were either inhibited or were indicated with colourless to bluish colouration.

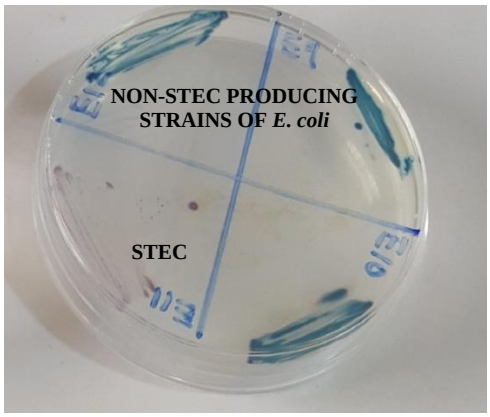

Fig. 1: Shiga-Toxin Producing *E. coli* on Chromagar STEC Base.

2.4. Antibiotic sensitivity profile

The antibiogramic activities of the isolates were determined as previously reported (Iyekhoetin et al., 2011). Briefly, bacterial isolates were inoculated into sterile nutrient broth in test tubes and incubated for 24hrs at 37^0C. After 24hrs isolates were streaked on already solidified Muller Hinton agar plates. Antibiotics disk were immediately placed on the streaked agar plates with forceps which were then incubated for 24hrs. Zone of inhibition indicated sensitivity of the isolates to the antibiotics, while absence of a clear zone indicated resistance of the organisms to the antibiotics.

3. Results and discussion

Shiga-toxin producing *Escherichia coli* (STEC) and other enterobacteriaceae are well known human pathogens globally. The sources of these pathogen of public health concern include beef, meat product, chicken, ready-to-eat salad etc. Fresh meat and meat product have previously been screened for the presence of STEC (Olatoye et al., 2012; Reuben and Gyar, 2015; Kabiru et al., 2015; Enabulele and Uraih, 2009). However, the occurrence of STEC in RTE salad has gained little or no attention especially in developing countries. To this end, the current study was aimed at investigating the current situation in Benin City, Edo State, Nigeria.

The results of the current study showed that RTE salad sold in Benin City, Edo State, Nigeria, are sources of human exposure to pathogenic microorganisms. All RTE salad samples obtained from both markets (Oba and Eki-Osa) had mean coliform and mean enterobacteriaceae counts ranging from 0.9 to 7.1 x 10^6 and 0.2 to 12.2 x 10^6cfu/mL respectively (Table 1).

Table 1: Mean Coliform Counts (MCCs) and Mean Enterobacteriaceae Counts (MECs) Of Ready-To-Eat Salad

SALAD SAMPLES	Oba Market (x 10^6 cfu/ml)		Eki-Osa Market (x 10^6 cfu/ml)	
	MCCs	MECs	MCCs	MECs
1	5.2	2.5	5.6	2.6
2	3.9	2.4	2.9	4.5
3	7.1	9.2	4.5	3.2
4	6.3	2.8	2.1	4.1
5	5.8	7.6	0.9	1.4
6	2.0	0.2	1.8	1.9
7	5.0	1.5	2.6	3.2
8	1.7	12.2	3.4	4.8
9	3.4	6.1	2.8	0.6
10	1.3	4.3	2.4	9.4
11	1.8	2.1	2.0	2.0
12	2.4	3.0	3.1	1.6

A total of thirty-one different strains of *E. coli* were obtained from all ready-to-eat salad from all sampling locations. Two of these *E. coli* strains were further confirmed to be STEC (Figure 1). Interestingly, both *E. coli* strains habouring the shiga-toxin gene obtained in this study were from the same sampling market (Oba market). Other Enterobacteriaceae present in all salad samples included *Klebsiella* spp, *Enterobacter* spp, *Serratia* spp, *Salmonella* spp and *Proteus* spp.

The antibiogramic activities of the isolates showed that all bacterial isolates were resistant to augmentin and amoxicillin. The cumulative antibiotic resistant profile of the isolates is shown in Table 2. The highest resistance (100%) was obtained with amoxicillin and augmentin, while the least was obtained with ciprofloxacin

and ofloxacin [11.1%] (Table 2). The percentage resistance for the shiga-toxin producing *E. coli* was (60%). Also the suspected enterobacteriaceae, *Serratia* showed resistance to all the antibiotics used.

Table 2: Antimicrobial Susceptibility of Bacterial Isolates to Commonly Used Antibiotics

Isolates / Antibiotics	1	2	3	4	5	6	7	8	9
	ZONES OF INHIBITION (mm)								
CN	R	15	15	10	15	15	R	R	20
AU	R	R	R	R	R	R	R	R	R
AM	R	R	R	R	R	R	R	R	R
CPX	25	30	20	20	20	20	R	25	20
SP	20	20	20	15	20	25	R	20	R
CH	25	R	20	R	20	25	R	R	R
SXT	R	R	15	R	25	30	R	R	R
S	R	R	20	R	10	25	R	R	R
OFX	25	20	20	10	15	15	R	15	20
PEF	R	20	15	20	15	20	R	15	20
% Resistance	60	50	20	50	20	20	100	60	60

Key: CPX: Ciprofloxacin; AM: Amoxicillin, AU: Augmentin; CN: Gentamicin; OFX: Ofloxacin; PEF: Pefloxacin; S: Streptomycin; SXT: Septrin; 1: *Klebsiella* spp 1; 2: *Klebsiella* spp 2; 3: *Proteus* spp; 4: *E. coli* 1; 5: *Enterobacter* spp; 6: *E. coli* 2; 7: *Serratia* spp; 8: *Salmonella* spp; 9: Shiga-toxin producing strain of *E. coli*.

The results of the current study showed that RTE salads sold in Benin City, Edo state, Nigeria are not free of coliform, enterobacteriaceae and STEC. The presence of coliform in ready- to- eat salad samples is an indication of faecal contaminants either from the food handlers, chopping boards or cutting knife (Oguwike et al., 2014).

The prevalence of *E. coli* strains in ready-to-eat salad was (100%), and is in keeping (although slightly higher) with the results of Enabulele and Uraih (2009), who reported the prevalence of *E. coli* in RTE vegetables (lettuce, cabbage, tomatoes) to be 83.33%. The presence of *Escherichia coli* in RTE salad is of concern as *Escherichia coli* are well known food-borne pathogen which is able to cause food-borne illness globally.

The presence of *E. coli* in salad and vegetables could be attributed to a number of factors. For example, a number of vegetable farmers in developing countries like Nigeria use cow dungs and poultry waste as manures, all of which have been reported as carrier of both faecal coliform and STEC. Another likely source of contamination with this organism is the vehicles on which the vegetables are transported to the various sales point. It has been reported that vegetables are transported using the same vehicles used in transporting cattle from one part of the country to the other, which could also be a source of concern.

The result obtained with STEC in this study is similar with the results of Puspanadan et al., 2013; Khatib and Khawaja 2015 and Somayeh et al., 2013. In one of these studies, Khatib and Khawaja (2015) found 4.8% (7 out of 145) of Lebanese fresh vegetables to contain STEC. The fresh vegetables included lettuce, tomato, cucumber and radish. Similarly, Somayeh et al., (2013) found 8% (8 out of 100) of ready to eat vegetables from a city in Iran to contain STEC. In both studies, the percentage occurrence of STEC was lower than that obtained in the current study. However, Puspanadan et al., (2013) found a slightly higher percentage (18.5) of STEC from Malaysia than that reported in the current study.

The result of this study is also contrary to the report of Enabulele and Uriah (2009), who reported zero incidence of STEC from vegetables sold in Benin City, Edo State, Nigeria.

The high occurrence of Enterobacteriaceae is supported by Sproston et al. (2006). These organisms are mainly found in the intestinal tracts of humans and animals, and are mostly pathogenic to humans. Faecal materials, decaying matter, contaminated water, selling environment and poor handling are some factors that could contribute to the high numbers of enterobacteriaceae.

In conclusion, the low level of sanitary practices amongst food handlers as well as lack of adequate data on outbreaks of infections could aid the spread of pathogenic organisms without detection. Hence, it is important to take seriously the isolation of these organisms in RTE salad from Benin City, Edo State, Nigeria. Consequently, it is recommended that food handlers wash food items using appropriate food grade anti-microbial as well as clean materials needed for the preparation of RTE salad prior to consumption. Finally, RTE salad should not be carried using the same containers used for fresh meat to avoid cross contamination.

4. Conflict of interest

The authors declare that there are no potential conflicts of interest

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