Occurrence of *Escherichia coli* O157:H7 in vegetables grown and sold in Lafia metropolis, Nigeria

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Fresh vegetables have been implicated in several outbreaks of food-borne disease caused by *Escherichia coli* O157:H7, an emerging pathogen of increasing public health significance in most parts of the world. This present study investigated the occurrence of *E. coli O157:H7* in vegetables grown and sold in Lafia metropolis, Nigeria. Forty (40) vegetable samples (spinach, cabbage, cucumber and bitter leaf) used in this study were obtained from markets and farms in Lafia metropolis. Strains of *Escherichia coli O157:H7* were isolated bacteriologically by means of cultural techniques (involving enrichment on modified E.C broth supplemented with novobiocin (mEC+n) and selective plating on Cefixime-Tellurite Sorbitol McConkey Agar), biochemical characterization (Microbact 12E) and serological assays (Oxoid diagnostic kit, latex R30959601). The results obtained from this study indicated that 7 (17.5%) of the vegetables examined were contaminated with *E. coli O157:H7*. The occurrence of *E. coli O157:H7* in cabbage, cucumber, spinach and bitter leaf was 20% (2), 10% (1), 30% (3) and 10% (1) respectively. The highest occurrence of *E. coli O157:H7* observed from this study was in spinach 30% (10) while the least was recorded in bitter leaf 10% (1). Samples obtained from farms and markets indicated 25% (5) and 10% (2) occurrence of *E. coli O157:H7*. This revealed that samples from farms were more densely contaminated than samples obtained from the markets. There was no statistically significant relationship (P>0.05) between the occurrence of *E. coli O157:H7* in the various vegetables examined and the sampling sites. The presence and survival of *E. coli O157:H7* in vegetables call for public health concern and a need for surveillance of *E. coli O157:H7* in vegetables both in urban centres and rural areas.

**Key words:** *Escherichia coli* O157:H7, vegetables, occurrence, public health.

**INTRODUCTION**

One of the most significant food-borne pathogen that has gained increased attention in recent years is the *Escherichia coli* O157:H7 (Schlundt, 2002). *E. coli O157:H7* was first recognized as food pathogen in 1982 (Riley *et al*., 1983). Since then, it has become a pathogen of major concern in both food and dairy industries, and to the public, because of its ability to cause severe illness, in particular, haemorrhagic colitis, haemolytic uremic syndrome and thrombotic thrombocytopenic purpura (Abdul-Raouf *et al*., 1993). Vegetables are exceptional dietary source of nutrients, micronutrients, vitamins and fibre for human beings hence vital for health and fitness (Tsado and Adesina *et al*., 2013).

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Well balanced foods rich with vegetables are especially valuable for their ability to prevent deficiencies of vitamin C and vitamin A and also reduce the risk of several diseases (Kalina and Gupta, 2006). Vegetables can become contaminated with pathogenic microorganisms from harvesting equipments, transport containers and domestic animals (Uzeh and Adepoju, 2013). The pathogenic microorganisms which reside in 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). Outbreak of food infections associated with consumption of ready-to-eat vegetables has been increasing in recent times (Beuchat, 1998). 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 mortality associated with E. coli O157:H7 infection therefore, make its presence in any food materials a serious public health concern as most of the outbreaks recorded has been traced to consumption of foods contaminated with E. coli O157:H7 strain (Hussein, 2007).

Nigeria is a country where agriculture is the most important sector of the economy. About 33% of Nigeria’s land area is under cultivation and agriculture contributed 32% to Gross Domestic Product in 2001 with exports totaling 323.5 million dollar. Urban farming is an emerging phenomenon in Nigerian cities of which vegetables cultivation is known to be the most widely spread. This is probably because of its contribution to balanced diet such as reduced risk for some forms of cancer, heart diseases, stroke and other chronic diseases (Ernest et al., 2011). Over the past two decades, consumption of fresh produce has increased (Olaimat and Holley, 2012). Consumers’ awareness of health aspects of vegetables and fruits have increased and consumers are more concerned about eating habits and staying healthy.

To the best of our knowledge there is no any documented study on the occurrence of E. coli O157:H7 on vegetables grown and sold in Lafia metropolis. Therefore, this investigation is of enormous importance as it would contribute to the understanding of the epidemiology of the infection caused by this pathogen. This study therefore, sought to determine the occurrence of E. coli O157:H7 grown and sold in Lafia metropolis, Nigeria.

MATERIALS AND METHODS

Study Area

Lafia is a town in central Nigeria. It is the capital city of Nasarawa State and has a population of 330,712 inhabitants according to the census result. Lafia has latitude of 8.4917°N and longitude of 8.5167°E.

Sampling Collections

A total of 40 vegetable samples comprising of 10 spinach (Spinacia oleracea), 10 cabbage (Brassica oleracea), 10 bitter leaf (Vernonia amygdalina) and 10 cucumber (Cucumis sativus) were collected from various markets and farms in Lafia metropolis. All samples were collected in sterile plastic bags, labeled and transported to the laboratory for analysis in ice pad.

Enrichment

10g of each vegetable sample was shredded using a sterile knife and added to 90ml modified E.C broth supplemented with novobiocin (mEC+n), and incubated at 37°C for 24hours.

Selective Plating and Identification of E. coli O157:H7 colonies

A loopful of the enriched samples were streaked onto Tellurite-Cefixime Sorbitol MacConkey agar (CT-SMA) plates and incubated at 37°C for 24 hours. A typical E. coli O157:H7 appeared as a non-sorbitol fermenter colony (NSFC) which is characterized as having a slightly transparent, almost colourless 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°C for 24hours. This was then refrigerated for further biochemical and serological analysis.

Biochemical test

The isolates were characterized biochemically using Microbact 12E (MB1130A+, Oxoid) according to the manufacturer’s instruction. Identification was done following a series of biochemical tests: Voges-Proskauer reactions, indole, citrate, urea hydrolysis, hydrolysis of O-nitrophenyl-β-d-galactopyranoside (ONPG), production of indolepyruvate by deamination of tryptophan, sugar (glucose, mannitol and xylose) fermentation, nitrate reduction, H2S production, lysine and ornithine decarboxylase.

Serological test

E. coli positive colonies were serologically confirmed by using E. coli O157:H7 latex agglutinations assay (R30959601, Oxoid), containing latex particles coated with antibodies specific for E. coli O157 and E. coli H7
Table 1. Percentage occurrence of E. coli O157:H7 from vegetable samples

<table>
<thead>
<tr>
<th>Vegetables</th>
<th>No. Examined</th>
<th>No. Positive</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabbage</td>
<td>10</td>
<td>02</td>
<td>20</td>
</tr>
<tr>
<td>Cucumber</td>
<td>10</td>
<td>01</td>
<td>10</td>
</tr>
<tr>
<td>Spinach</td>
<td>10</td>
<td>03</td>
<td>30</td>
</tr>
<tr>
<td>Bitter leaf</td>
<td>10</td>
<td>01</td>
<td>10</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>40</strong></td>
<td><strong>07</strong></td>
<td><strong>17.5</strong></td>
</tr>
</tbody>
</table>

Table 2. Occurrence of E. coli O157:H7 in vegetables from sampling sites

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>No. Examined</th>
<th>No. Positive</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm</td>
<td>20</td>
<td>05</td>
<td>25</td>
</tr>
<tr>
<td>Market</td>
<td>20</td>
<td>02</td>
<td>10</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>40</strong></td>
<td><strong>07</strong></td>
<td><strong>17.5</strong></td>
</tr>
</tbody>
</table>

antigen. Isolates were tested separately with anti-O157, and H7 antisera. Identification of E. coli O157:H7 was carried out following the manufacturer’s instruction, hence colonies that agglutinated to the separate antisera were considered to be E. coli O157:H7.

RESULTS

Out of the 40 vegetable samples examined for E. coli O157:H7, 7 were contaminated with E. coli O157:H7, having an occurrence rate of 17.5%. The occurrence of E. coli O157:H7 in cabbage, cucumber, spinach and bitter leaf is 20% (2), 10% (1), 30% (3) and 10% (1) respectively (Table 1). The highest occurrence rate observed from this study was in spinach 30% (10) while the least was recorded in bitter leaf 10% (1). Samples obtained from farms and market indicated the occurrence rates of 25% (5) and 10% (2) (Table 2). Samples from farms were more densely contaminated than samples obtained from the markets.

DISCUSSION

This study revealed that culturable E. coli O157:H7 were present in vegetable samples examined from Lafia metropolis, Nigeria, with an occurrence of 17.5%. The low 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), Benard, (2008) and Enabulele et al. (2009) who reported the occurrence rates of 18.2%, 21.66% and 19.5% respectively. Despite the low occurrence of E. coli O157:H7 in vegetables as recorded from this study, pre- and post-harvest handling of vegetables in Lafia 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; Wilshaw et al., 1994). The presence of E. coli O157:H7 indicates recent contamination by faecal matter and possible presence of other enteric pathogens known to be causative agents of food borne gastroenteritis and bacterial diarrhoea disease (Jiwa et al., 1981; Adebayo-Tayo et al., 2012). The isolation of this pathogen in vegetables therefore poses food safety problem. Farmers use bovine manure and untreated water on their farms to grow vegetables and the vegetables are in some cases sold in open-air markets. These vegetables are displayed either on top of wooden racks or on the ground in these open-air markets. Studies have shown that E. coli O157:H7 can get internalized in vegetables through contaminated irrigation water and soil (Solomon et al., 2002). However, the occurrence of this pathogen in vegetables may have been a direct reflection of sanitary quality of the cultivation water, harvesting, transportation, storage, processing and poor handling practices (Beuchat, 1998; Josefa et al., 2005). Separate studies have confirmed that fresh produce such as lettuce, alfalfa sprouts and cabbage may become contaminated in the farms or during transportation and marketing (Como–Sabetti et al., 1997; Ackers et al., 1998; Wojtkunski et al., 1999).

In 2003 and 2004, Mukherjee et al. (2004) in a microbiological survey of fresh produce with E. coli O157:H7 being one of their target pathogenic bacteria failed to isolate the bacteria from the vegetables. Another study in Norway, which attempted to detect E. coli O157:H7 in lettuce did not succeed (Loncarevic et al., 2005). An investigation by Sagoo et al. (2001) that evaluated the microbiological quality of ready-to-eat vegetables, which included 86 carrots, mushrooms, cherry, pepper and alfalfa sprouts and 3200 samples of broccoli, cabbage, celery, lettuce, radish and others did not reveal the presence of E. coli O157:H7 in any of the vegetable samples they studied. This can be explained in
Occurrence of Escherichia coli O157:H7 in vegetables grown and sold in Lafia metropolis, Nigeria

part by assuming either that these bacteria are absent from the vegetables that were studied or that they are present in very low counts. However, recently E. coli O157:H7 were isolated from spinach that caused an outbreak in the US, which infected approximately 81 individuals (FDA News, 2006a, b).
The specific vegetable with the highest occurrence of E. coli O157:H7 as recorded from this was spinach 30% (3) followed by cabbage 20% (2). This could be because it is readily cultivated and grown in this region. Also, manure from cattle and other animals are normally used in such farms. This is in consonance with various studies which indicated that cattle are the primary reservoir of E. coli O157:H7. Cabbage heads are often split into halves to meet customers’ financial affordability. The cut cabbage surfaces in most cases are not covered to protect them from contaminants in the surrounding environments. These practices expose the vegetables to open environments thus they may be easily contaminated by air-borne pathogens. Bitter leaf had the least occurrence rate of 10% (1) from this study. Bitter leaf plant is normally taller and far from the ground. Even when manure is added to the farm the rate of E. coli O157:H7 contamination of the leaves is very minimal due to the distance of the leaves from the ground.

However, contamination of vegetables with pathogenic E. coli O157:H7 has rarely been reported in the literature mostly from the African continent. Although, in western countries there have been studies on microbiological qualities of vegetables the majority of these studies have often been unsuccessful in isolating E. coli O157:H7 (Martinez et al., 2000; Mukherjee et al., 2004).

With respect to sampling sites, samples obtained from farms recorded a greater occurrence (25%) of E. coli O157:H7. This could be as a result of waste water used on the vegetables. Often, untreated seawage water and animals’ dung are used on farm lands, and the unhygienic condition of the area where the vegetables were produced. These support the claim of Beuchat and Rey, (1997) and Beuchat (1998) that the presence of many pathogens in the soil was thought to be from historical application or environmental presence of faeces or untreated sewage. Also, pathogens existing in the soil or water can be the source of both pre and post-harvest contamination. These higher bacterial loads in urban area might also not be unconnected with the life style (comprising of throwing rubbish anyhow) thereby quickly increasing the concentration of pollution in the cities (Tsado et al., 2013). Harris et al. (2003) reported that leafy vegetables when eaten raw can transmit contamination from farms to consumers. Thus, this higher bacteria load on leafy vegetables calls for caution, because many people use vegetables for medicine where they only squeeze out the juice and drink it raw, as well as in salads.

Also, the water used for washing vegetables is another possible source of cross contamination. Vegetables are usually washed and immersed together before packaging process. According to Wang and Doyle, E. coli O157:H7 is able to survive in water for a long period (especially in cold temperatures) (Wang and Doyle, 1998). In such conditions the chance of E. coli O157:H7 contamination will increase.

CONCLUSION

Contamination of vegetables with E. coli O157:H7 as was revealed in this study make stronger the need of surveillance for E. coli O157:H7 in Lafia metropolis and Nigeria in general. The need for microbial assessment of these vegetables for production of salads and for other use cannot be over emphasized to reduce possible contamination. Consumers should be educated about the potential risk of consuming uncooked fresh vegetables. Regulatory and educational efforts are needed to improve the safety of fresh farm vegetables that are intended for use as ready-to-eat vegetables.

REFERENCES


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