



Occurrence of Pathogenic *Escherichia coli* O157:H7 in Vegetables, Fruit and Milk in Selected Areas of Sabon Gari LGA, Kaduna State, Nigeria: A Short Communication

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ABSTRACT

Fresh fruits, vegetables, and milk are common sources of microbial food-borne diseases. This study aimed to assess the prevalence of pathogenic *E. coli* O157:H7 in these food items sold in Samaru Ultra-Modern Market and fresh milk from National Animal Production Research Institute, Zaria, Nigeria. A total of 50 samples each of fruits, vegetables, and milk were collected for analysis. For vegetable, fruit and milk, *E. coli* was isolated using Eosin-Methylene Blue agar (EMB), with a 66% (33/50), 68.0% (34/50), 34% (17/50) prevalence respectively. The biochemical characterization confirmed a total of 33, 28 and 14 *E. coli* isolates for vegetables, fruit and milk respectively. The Cefixime-Tellurite Sorbitol MacConkey's agar (CT-SMAC) identified 15 samples of vegetables, 18 samples for fruits and 12 samples for milk. Of all, ten different vegetables were studied, cabbage 100% (5/5) and lettuce 100% (5/5) showed the highest prevalence of pathogenic *E. coli* O157:H7 detected using serology. Fruits- 36% (18/50) and milk- 24% (12/50) collected from the specified area in Sabon gari LGA, were also contaminated with enterohaemorrhagic *E. coli* O157:H7. There was no significant association ($p = 0.4244$) between pathogenic *E. coli* O157:H7 and the food items (vegetable, fruits and milk). These findings highlight the risk of microbial contamination in these food items, necessitating proper hygiene practices and, in the case of milk, pasteurization to mitigate health risks for consumers.

ARTICLE INFO

Article History

Received: October, 2024

Received in revised form: November, 2024

Accepted: January, 2025

Published online: March, 2025

KEYWORDS

Enterohaemorrhagic *E. coli* O157:H7,
Vegetables, Fruit, Milk

INTRODUCTION

Escherichia coli is a commensal bacterium with strains that have acquired specific virulence factors by means of mobile genetic elements, such as plasmids, transposons, bacteriophages, and pathogenicity islands, and have evolved into pathogenic *E. coli* [1]. Enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC) and enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC) and Enterotoxigenic *E. coli* (EAEC) are among the pathogenic strains of *E. coli* [2].

E. coli O157:H7 is a member of the EHEC group that is one of the most dangerous foodborne pathogens as it can cause grievous illnesses such as inflammation, abdominal pain, diarrhea, hemolytic uremic syndrome, hemorrhagic colitis, and even death, especially in young, old and immunocompromised individuals [3]. These bacteria are transmitted via fecal-oral route and its versatility and adaptability makes it very commonly found in water, soil, and food [4]. These concerned pathotypes of *E. coli* cause significant public and environmental health

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challenges. Their minimal infectious doses, high infectivity rate, and transmission through many channels makes them a pathogenic bacteria with public health significance [5, 6].

Water and soil can be sources of *E. coli* contamination to plant produce [7, 8] and the bacteria is a common cause of poor milk microbial quality often resulting from unhygienic milk handling [9]. Furthermore, the main contamination factors in fresh vegetables and plant produce includes preharvest practices such as irrigation, manuring, harvesting, seasonal impact and post-harvest practice such as transport and storage [10]. Also, foodborne disease pathogens could contaminate milk from the udders of cows from activities of milkers, unhygienic milking facilities, faecal materials from the milking environment [11] as well as stages between processing and final consumption [12]. In most developing countries, including Nigeria, there is usually no adequate hygiene practices in handling and selling plant produce in markets. Contaminated water, unsanitary storage conditions, and inadequate washing of fruits and vegetables are key factors that can significantly increase the risk of transmission of pathogenic *E. coli*. In addition, the low- input transhumance pastoral systems which is the country's major source of milk production by dairy cows is another route of transmission for these bacteria [13] and this could lead to harm inadvertently. A study reported the presence of

pathogenic *E. coli* in fresh spinach collected from Samaru market [14], therefore; it is necessary to conduct research on contamination of pathogenic *E. coli* 0157:H7 from vegetables, fruits and milk from Samaru Ultra-Modern Market and National Animal Production Research Institute, Sabon gari L.G.A, Zaria.

METHODOLOGY

Sample collection

The study was conducted in Samaru central market and National Animal Production Research Institute, Zaria, Sabon Gari Local Government Area of Kaduna State (Fig 1.0). A total of fifty (50) vegetables and fifty (50) fruits were collected from the randomly sampled grocery stores, and street vendors in several areas in Samaru central market (Longitude and Latitude). Also, a total of fifty bottles of fresh milk from dairy cattle were collected from female Fulani trader (25) in Samaru Market and National Animal Production Research Institute (Longitude and Latitude) in Zaria. The vegetable samples - *Brassica oleracea*, *Daucus carota*, *Corchorus olitorius*, *Spinacia oleracea*, *Ocimum gratissimum*, *Lactuca sativa*, *Abelmoschus esculentus*, *Talinum triangulare*, *Allium cepa*, *Vernonia amygdalina* and fruit samples – *Citrus sinensis*, *Musa paradisiaca*, *Carica papaya*, *Citrullus lanatus* and *Malus pumila* were collected.

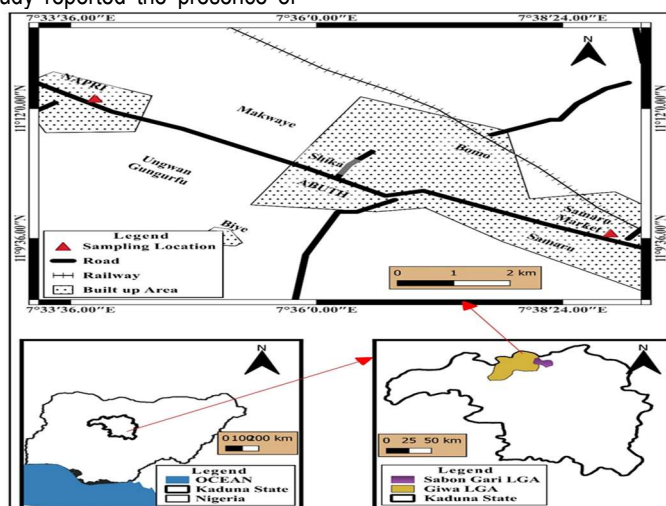


Fig 1.0: Map Showing Samaru Market and NAPRI in Sabon gari LGA.

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Isolation and identification of *E. coli* in Vegetables, Fruits and Milk

The leaves of the different vegetables and surface of fruits collected from the Samaru market were washed thoroughly with distilled water and the washing was placed in universal bottles [14]. Five (5) millilitres of milk, vegetable and fruit washing were inoculated in sterilized Brain Heart Infusion (BHI) medium (5 ml) and incubated at 37°C for 24 hours. A loopful of the positive BHI culture was streaked onto Eosin Methylene Blue (EMB) agar using the streak plate method. The EMB plate was incubated at 37°C for 24 hours. The EMB agar was observed for colonies with metallic green sheen which was considered as *E. coli*. Samples with colony resembling *E. coli* was picked up from the EMB plates and biochemically tested using Citrate test, Sulphate Indon Motility test (SIM), Triple Sugar Ion test, and Methyl Red-Voges Proskauer test (MRVP) and Urea test [15]. Colonies positive for biochemical test were streaked on Cefixime-Tellurite Sorbitol Macconkey Agar and colonies without pink colours confirm the presence of *E. coli* [16].

Agglutination test for *E. coli* O157:H7

A standard method was used for the agglutination test specific to *E. coli* O157:H7 [17, 18]. All cultures that have been isolated and identified as *E. coli* bacteria from the (CT-SMAC) test [19] were identified by agglutination using monospecific O157:H7 antiserum for identification as *E. coli* O157:H7. Two drops of overnight broth and two drops of *E. coli* antiserum were placed on a clean slide, emulsified, and shake gently. The appearance of particles shows presence of pathogenic *E. coli* O157:H7.

Data analysis

The prevalence rate for vegetable, fruits and milk was expressed in percentages of the total number of *E. coli* that was sampled. Chi square was used to test for significant association between the pathogenic *E. coli* O157:H7 and the sampled vegetables, fruits and milk. The value of

$p < 0.05$ was considered significant. The analysis was done using R version 4.2.1.

RESULTS

The Overall Prevalence of *E. coli* in Vegetables, Fruits and Milk

The prevalence of *E. coli* in vegetables, fruits, and milk sampled from Samaru market and NAPRI is presented in Table 1, showing that among the 50 vegetable samples, 5 cabbages, 4 onions, 3 spinach, 4 jute leaves, 3 bitter leaves, 5 lettuces, 2 scent leaves, 1 water leaf, 2 okras, and 4 carrots were positive for EMB agar; among the 50 fruit samples, oranges, bananas, paw-paws, watermelons, and apples were positive for EMB agar; and among the 50 milk samples, 13 from Samaru market and 14 from NAPRI were positive for EMB agar. The number of individual samples positive for biochemical test were same as the EMB agar. For the CT-SMAC agar, 5(100) cabbage, 1(33.3) spinach, 4(100) jute leaf, 5(100) lettuce, were positive among the vegetables sampled, 4(100) oranges, 5(100) banana, 4(50) paw-paw were positive for fruits while 10 out of 11 milk samples from Samaru market and 2 out of 3 milk samples from NAPRI were positive for *E. coli*.

The Agglutination test for *E. coli* O157:H7 in Vegetables, Fruits and Milk

The agglutination test showed samples positive for *E. coli* O157:H7 and a total of 15 vegetable, 18 fruits and 12 milk samples were positive for *E. coli* O157:H7 (Table 2). The pathogenic bacteria were observed in all samples of 5(100) cabbage and 5(100) lettuce. Also, the fruits sampled for this study such as Orange (4), Banana (5), Paw-paw (4), Water melon (5) were all contaminated with *E. coli* O157:H7 except the Apple (0). All milk samples positive for CT-SMAC agar irrespective of the location where all positive for the agglutination test. In this study, there was no significant association between the presences of *E. coli* O157:H7 and the type of food (vegetables, milk, and fruits).



Table 1: Prevalence of *E. coli* detected in vegetables, fruits and milk from Samaru market and NAPRI

Sampling point	Group	Types	Number of samples examined	Number of positive samples for EMB	Number of positive samples for biochemical test	Number of positive samples for <i>E. coli</i> on CT-SMAC agar
Samaru market	Vegetables	Cabbage	5	5(100)	5(100)	5(100)
		Onion	5	4(80)	4(100)	0(0)
		Spinach	5	3(60)	3(100)	1(33.3)
		Jute leaf	5	4(80)	4(100)	4(100)
		Bitter leaf	5	3(60)	3(100)	0(0)
		Lettuce	5	5(100)	5(100)	5(100)
		Scent leaf	5	2(40)	2(100)	0(0)
		Water leaf	5	1(20)	1(100)	0(0)
		Okra	5	2(40)	2(100)	0(0)
		Carrot	5	4(80)	4(100)	0(0)
		Total	50	33(66)	33(66)	15(45.5)
	Fruits	Orange	10	7(70)	4(57.1)	4(100)
		Banana	10	7(70)	5(71.4)	5(100)
		Paw-paw	10	8(80)	8(100)	4(50)
		Water melon	10	8(80)	7(87.5)	5(71.4)
		Total	50	34(68)	28(82.3)	18(62.28)
National Animal Production Research Institute	Milk		25	13(52)	11(84.61)	10(90.9)
	Milk		25	4(16)	3(75)	2(66.6)
	Total		50	17(34)	14(82.35)	12(85.71)

Table 2: Prevalence of *E. coli* O157:H7 detected in vegetables, fruits and milk from Samaru market and NAPRI

Sampling point	Group	Types	Number of samples examined	Number of sample for <i>E. coli</i> O157:H7	p-value
Samaru market	Vegetables	Cabbage	5	5(100)	0.4244
		Onion	0	0(0)	
		Spinach	1	1(100)	
		Jute leaf	4	4(100)	
		Bitter leaf	0	0(0)	
		Lettuce	5	5(100)	
		Scent leaf	0	0(0)	
		Water leaf	0	0(0)	
		Okra	0	0(0)	
		Carrot	0	0(0)	

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Sampling point	Group	Types	Number of samples examined	Number of sample for <i>E. coli</i> O157:H7	p-value
National Animal Production Research Institute	Fruits	Total	15	15(100)	
		Orange	4	4(100)	
		Banana	5	5(100)	
		Paw-paw	4	4(100)	
		Water melon	5	5(100)	
		Apple	0	0(0)	
		Total	18	18(100)	
	Milk		10	10(100)	
	Milk		2	2(100)	
	Total		12	12(100)	

Note: A significant association between *E. coli* O157:H7 and vegetables, fruits and milk was set at $p < 0.05$

DISCUSSION

Escherichia coli is a well-studied member of the Enterobacteriales family that is commonly host-associated while also capable of thriving in diverse environments including water and soil. It is both a common commensal and pathogen, responsible for intestinal and extraintestinal infections in humans and animals [20, 21], respectively referred to as intestinal pathogenic *E. coli* (IPEC) and extraintestinal pathogenic *E. coli* (ExPEC), and a contaminant of agricultural crops and food products [22, 23]. Large volumes of water are used during harvest and post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs as well as during fresh-cut/freeze value-added operations, distribution and end-user handling of these food products. Poor quality of the water used for processing operation and the hygiene of people in charge of the operations could contribute to the accumulation of microbial hazard [24]. This is also the case in milk production as poor cleaning, sanitation, animal health, and milk hygiene are the major risk factors increasing foodborne outbreak [25].

The prevalence of *E. coli* occurred the most in vegetables and in a descending order for cabbage, lettuce, jute leaf and spinach. Milk and fruit samples were also contaminated with *E. coli* but the number were less than vegetables when

the total samples examined are considered. A study conducted in Zaria also observed a higher prevalence of *E. coli* in vegetables like lettuce than in fruits such as watermelon [26]. Also, Fathuddin et al. [27] identified the presence of *E. coli* in fresh milk and fermented milk products in Zaria. The presence of *E. coli* in these samples indicates the possibility of faecal contamination from manures and inferior post-harvest washing by processors to remove soil and debris [28]. It may also be due to cross contamination by the handlers through poor hand washing, or contamination of utensils and preparation surfaces [29].

The result of this present study also revealed the contamination of vegetables, fruit and milk with *E. coli* O157:H7. Although, there was no significant association between *E. coli* O157:H7 and the food items ($p = 0.4244$), the total vegetable sample contaminated with *E. coli* O157:H7 was higher per the number of samples examined for each category, for instance, 100% of cabbage and lettuce were contaminated with the pathogenic bacteria when compared with other fruits and milk samples. Incidentally, these are among the most consumed ready-to-eat fresh vegetables. It has been observed that fruits and vegetables that get in contact with the soil have a higher risk of being contaminated, especially if the soil is contaminated with animal/human waste or manure that is not well-composted [30]. This is in

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contrast with a study conducted in Akwa Ibom where *E. coli* isolated from fruits and vegetables in the study were subjected to serological test to detect *E. coli* O157 serogroup and no sample was positive for the O157 serogroup [31].

Contamination of fruits and vegetables with enterohaemorrhagic *E. coli* O157:H7 may occur when cattle and perhaps other ruminants inadvertently enter the farms, or when improperly composted animal manure has been applied as fertilizer [30, 32]. To reduce the risk of contamination of produce through the use of animal composted manure, certified organic methods require that the manure applied to fields must be properly composted before application. Also, harvesting of the farm produce for human consumption should be delayed for at least 90 days after the application, for edible portions of the crop not in direct contact with the soil and 120 days for edible portions in direct contact with the soil [33]. Proper hygiene practices should be observed in the case of milk pasteurization and handling to mitigate health risks for consumers.

CONCLUSION

Enterohaemorrhagic *E. coli* O157:H7 is a pathogenic bacterium that can be transmitted in the environment from sources such as animal faeces to the soil, food and water. It is associated with a debilitating disease condition in human and the study has revealed the contamination of vegetables, fruits, and milk sampled in selected areas of Sabon Gari LGA, Zaria with *E. coli* O157:H7.

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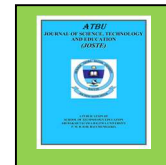
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