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Characterization of Enterohemorrhagic *Escherichia coli* Isolated from Leafy Vegetables Sold in Major Vegetables Outlets Within Katsina Metropolis

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ABSTRACT

Food-borne diseases caused by different strains of pathogenic *Escherichia coli* such as *E. coli* O157:H7have been a threat in both developed and developing countries, as such rapid identification and characterization of this pathogen is important for food hygiene management and prompt epidemiological investigations. The present study characterized enterohaemorrhagic *Escherichia coli* (*E. coli* O157:H7) isolated from cabbage, lettuce, and spinach sold at Lambun sarki and Central market within Katsina metropolis. Four samples of each vegetable were purchased from the two locations. Samples were processed, followed by isolation and characterization of *Escherichia coli* using standard microbiological procedures. Among the twenty-four (24) samples analysed, four *Escherichia coli* were isolated. Only one of the isolated *Escherichia coli* shows negative sorbitol fermentation in sorbitol MacConkey agar indicating *Escherichia coli*O157:H7 while others were positive. This indicated that some samples were contaminated with *Escherichia coli* and are not fit for human consumption going by World Health Organisation's recommendations and guidelines. This suggests the observance of hygienic protocols during harvest, handling and at point of sales.

Keywords: *E. coli* O157:H7, vegetables, food borne disease, pathogen

INTRODUCTION

Enterohemorrhagic *Escherichia coli (*EHEC) is one of the Shiga-toxin producing strain of *E. coli* and one amongst the bacteria associated with various food borne diseases such as thrombotic thrombocytopenic purpura, haemolytic-uremic syndrome, haemorrhagic colitis, and watery and/or bloody diarrhoea (Wang *et al*., 2014). This pathogen is capable of surviving in various environmental conditions, and this is due to a variety of mechanisms amongst which is its ability to adhere to surfaces and internalization in fresh products; the key virulence factors for the pathogenesis of this organism are the Shiga-toxin, intimin, and enterohemolysin (Wu *et al*., 2011). In recent years, food-borne diseases caused by different strains of enterohemorrhagic *E. coli* (EHEC) such as the *E. coli* O157:H7 have been a threat in both developed and developing countries; several studies have shown that in industrialized countries, about one third of the population is affected by food borne diseases each year, and the problem is probably even more in developing countries. In fact, *E. coli* O157:H7 alone has caused illnesses in more than 30 countries in different continents (Benjamin *et al*., 2018).

Minimally processed and ready to eat leafy vegetables such as cabbage, lettuce, and spinach are nowadays considered as important part of a

healthy diet; yet, they are still considered some amongst the vegetables mostly associated with bacterial infections (Jay-Russell *et al*., 2014; Benjamin *et al*., 2018). In the past decades, several outbreaks of *E. coli* O157:H7 infections have been associated with the consumption of raw vegetables, potatoes, radish sprouts and leaf lettuce (Kim *et al*., 2014; Dahiru *et al*., 2015). These vegetables (used for preparing salads) are usually contaminated with *E. coli* O157:H7 from soil fertilized by animal manure, human waste, surface water irrigation, or water mixed with sewage; the contamination may also arise during the course of handling, collection, washing, slicing, soaking, processing, transportation, and packaging (Castro-Rosas *et al*., 2012; Luna-Guevara *et al*., 2019). The conventional processing and chemical sanitizing methods used by food vendors/industry do not guarantee total elimination of pathogens like *E. coli* O157:H7 (Abadias *et al*., 2012; Luna-Guevara *et al*., 2019).

Several research have been conducted on enterohaemorrhagic *E. coli* O157:H7 associated with diarrheic stools, beef ground meat, sheep and goats milk, cattle faecal materials, and drinking waters from wells (Alam *et al*., 2013: Saeed *et al*., 2013; Saeed and Ibrahim, 2013) but there is few information on *E. coli* O157:H7 from leafy vegetables consumed within Katsina metropolis. It is in view of this that this study aimed to characterize *E. coli* O157:H7 isolated from leafy vegetables sold within Katsina metropolis.

MATERIALS AND METHOD

Study Area

The sample locations were two major vegetable outlets (i.e Lambun Sarki and Central Market) within Katsina Metropolis. Central market is a market place where among other things, vegetables are sold. It is situated nearby to the bus stations central motor park and Kano motor park all in Katsina Metropolis with a latitude of 12^0 58' 16" N, and longitude of 7^0 36' 6" E. Lambun Sarki is a vegetable garden located in Kofar Marusa Katsina Metropolis of Katsina State-Nigeria with a latitude of 12^0 59' 18.60" N and longitude of 7^0 37' 1.86" E.

Sample Collection

A total number of 24 samples were purchased from Lambun Sarki and Central Market in Katsina metropolis, Katsina town, Katsina state, Northwest region of Nigeria. Four (4) samples each, of different vegetables namely: lettuce, cabbage, and spinach from each of the two aforementioned locations were aseptically collected in sterile polythene bags and transported to the biological science laboratory, Al-Qalam University Katsina, for bacteriological analysis.

Preparation of Stock Solution and Serial Dilution

An electrical blender was used to homogenize each of vegetable samples, 10 grams of the homogenized sample was measured and suspended in 90 ml of sterile distilled water to serve as a stock solution. One (1) ml of the stock culture was transferred to a test tube containing 9ml of sterile distilled water; a ten-fold serial dilution was made (Ben-David and Davidson, 2014).

Isolation of *Escherichia coli*

About 0.5ml from 10^{-3} and 10^{-5} serial dilutions were plated in duplicate onto prepared Mac-Conkey agar plates; the plates were incubated at a temperature of 37^0C for a period of 24 hours. After incubation, colonial characteristics were carefully observed; colonies that appeared pinkish (suspected to be *E. coli*) were sub-cultured onto a fresh Mac-Conkey agar plate and incubated at a temperature of 37^0C for a period of 24 hours (Benjamin *et al*., 2018).

Identification of Suspected *Escherichia coli* Isolates

A 24 hours culture of suspected *E. coli* isolates from fresh Mac-Conkey plate were inoculated onto Eosin Methylene Blue (EMB) agar plates, the plates were incubated at a temperature of 37^0C for a period of 24 hours for confirmation. Colonies that appeared greenish with metallic sheen were confirmed as *E. coli*. The confirmed isolates were sub-cultured on a Nutrient Agar plate, incubated at a temperature of 37^0C for a period of 24 hours to serve as stock culture (Dahiru *et al*., 2015; Benjamin *et al*., 2018).

Confirmation of *E. coli*

The *E. coli* isolates obtained above were further

identified by Gram reaction, cell morphology and biochemical characteristics.

Gram Staining

Gram staining was performed according to procedure described by Oyeleke and Manga (2008): Lawal and Yusuf (2021) and Fardami *et al*., (2022). The bacterial isolate was smeared using a drop of water on a clean, grease-free glass slide. As soon as the smudge had dried, it was touched to a flame. The smear was fixed, then stained with crystal violet for one minute and finally rined with water. After one minute, the slide was covered with lugol's iodine and washed with water. The stain was quickly decolorized with ethanol and washed with water. Then Safranin was applied, left for 30 seconds and washed off with water. The slide's reverse side was dusted with cotton wool and left to air dry the slide was analysed with microscope using oil immersion lens.

Indole Production Test

A loopful of each isolate was inoculated into a sterile tryphtophan broth and incubated for 37^0C for 48hrs. After incubation, 0.5ml of Kovac's reagent was added and shaken. This was examined for one minute Appearance of red ring in the reagent layer indicated indole production (Oyeleke and Manga, 2008: Fardami *et al*., 2022).

Methyl Red (MR)Test

A loopful of isolated was inoculated into a prepared glucose phosphate broth and incubated at 37^0C for 4days. After incubation, drops of methyl red indicator was added. The broth was shaken and examined. Appearance of ref color on the surface of the reagent layer indicated positive methyl red test (Oyeleke and Manga, 2008: Fardami *et al*., 2022).

Voges-Prokauer (VP) Test

To the part of 4days old culture above, 0.6ml 5% alpha naphthol was added and shaken. The test tubes were sloped and examined after 15 minutes. A red coloration indicated positive VP test (Oyeleke and Manga, 2008)

Citrate Utilization Test

This was carried out in Simmon's citrate medium. A loopful of the fresh isolate was inoculated asceptically on to the prepared Simmon's citrate medium and incubated for 24hrs at 370C. The medium was examined, and a change in colour from dark green to blue indicated positive utilization of citrate by the inoculated isolate (Chessbrough, 2002).

Screening *Escherichia coli* Isolates for Sorbitol Fermentation

A 24-hour culture of *E. coli* from nutrient agar plate was inoculated on prepared Sorbitol Mac-conkey agar plates. The inoculated plates were incubated at a temperature of 37^0C for a period of 24 hours. Colonies that appeared colourless (sorbitol negative) were identified (Saeed *et al*., 2013).

RESULT AND DISCUSSION

The colonial features of the bacteria presumptively detected are presented in Table 1. Pinkish and yellowish colonies were notable on Mac Conkey agar.

Table1: Detection of *Escherichia coli* on MacConkey Agar

Sampling Location	Sample Type	Sample Codes	Colonial Feature on MacConkey Agar
Central Market	Lettuce	CMLA	Pink colonies
		CMLB	Yellowish colonies
		CMLC	Pink colonies
		CMLD	Yellowish colonies
	Cabbage	CMCA	Pink colonies
		CMCB	Pink colonies
		CMCC	Yellowish colonies
		CMCD	Pink colonies
	Spinach	CMSA	Pink colonies
		CMSB	Yellowish colonies
		CMSC	Pink colonies
		CMSD	Pink colonies
Lambun Sarki	Lettuce	LSLA	Pink colonies
		LSLB	Yellowish colonies
		LSLC	Pink colonies
		LSLD	Yellowish colonies
	Cabbage	LSCA	Pink colonies
		LSCB	Pink colonies
		LSCC	Pink colonies
		LSCD	Pink colonies
	Spinach	LSSA	Yellowish colonies
		LSSB	Pink colonies
		LSSC	Pink colonies
		LSSD	Pink colonies

Key: CML = Central Market Lettuce, CMC = Central Market Cabbage, CMS = Central Market Spinach, LSL = Lambun Sarki Lettuce, LSC = Lambun Sarki Cabbage, LSS = Lambun Sarki Spinach

Growth characteristics of suspected *E. coli* isolates was analysed on eosin methylene blue agar. Only four isolates from the samples collected from central market gave exclusive character (green metallic sheen) of *E. coli* on Eosin Methylene Blue agar (EMB) (Table 2).

Table 2: Growth Characteristics of Suspected *E. coli* Isolates on Eosin Methylene Blue Agar

Isolates	Green Metallic Sheen on EMB Agar
CMCC	Positive
CMLA	Positive
CMLC	Positive
CMSB	Positive
CMCA	Negative
CMCB	Negative
CMCD	Negative
CMSC	Negative
CMSD	Negative
LSLA	Negative
LSCA	Negative
LSCB	Negative
LSCC	Negative
LSCD	Negative
LSSB	Negative
LSSC	Negative
LSCD	Negative

Table 3 shows the Gram reaction, morphology and biochemical tests of the isolates. All the isolates were Gram negative rods, positive to indole, methyl red, and citrate utilization tests but negative vp test and the identified species was *Escherichia coli*.

Table 3: Identification of the Isolates

Isolates	Gram Reaction	Morphology	Indole test	MR Test	VP	Citrate utilization test	Identified Species
CMCC	-ve	Rods	+ve	+ve	-ve	+ve	*E. coli*
CMLA	-ve	Rods	+ve	+ve	-ve	+ve	*E. coli*
CMSB	-ve	Rods	+ve	+ve	-ve	+ve	*E. coli*
CMLC	-ve	Rods	+ve	+ve	-ve	+ve	*E. coli*

Key: +ve = Positive, -ve = Negative

The number and percentage of *E coli* isolated from the samples are presented in Table 4 below. It was clearly shown that samples collected from central market were heavily contaminated with *E. coli* with the occurrence of 2(50.0%), 1(25.5 %), 1(25.5%) for lettuce, cabbage and spinach respectively. However, *E coli* was not isolated in the samples from the other location and this could be attributed to hygienic protocols employed by the sellers.

Table 4: Number and Percentage of *E. coli* Isolated from Leafy Vegetables

Location	Sample	No. of Sample	No. and % of Isolated *E. coli*
	Lettuce	4	2 (50.0%)
Central	Cabbage	4	1 (25,5%)
Market	Spinach	4	1 (25.5%)
	Lettuce	4	0 (0.0%)
Lambun	Cabbage	4	0 (0.0%)
Sarki	Spinach	4	0 (0.0%)
	TOTAL	**24**	**4 (`16.7%)**

Out of the 24 samples of vegetables namely: lettuce, cabbage, and spinach, which were examined in this study, only 4 (16.7%) were reported to be contaminated with *E. coli* which was lower than those (43%) reported by Olufemi and Oyedeji (2017) from Lagos, Nigeria. These low results can be due to several factors amongst which include the agricultural methods, samples size, and geographical area. The percentage of *E. coli* was 50% from lettuce, and 25% from cabbage and spinach, all of which were collected from Central Market. The high percentage of *E. coli* recorded from lettuce compared to cabbage and spinach may likely be due to the contamination during the course of handling, collection, washing, slicing, soaking or processing.

Negative Sorbitol fermentation is a characteristic of *E. coli* O157: H7, hence all isolates were tested for their ability to ferment sorbitol, and the result is presented in Table 5. Only *E. coli* isolated from cabbage sample of central market showed negative sorbitol fermentation.

Table 5: Sorbitol Fermentation of the Identified *Escherichia coli*

Sampling Location	Sample	Species		Sorbitol Fermentation	Strain	No. of *E. coli* Identified	No. and Percentage of *E. coli* O157:H7
	CMLA	*E. coli*		Positive		1	0(0%)
Central Market	CMLC	*E. coli*		Positive		1	0(0%)
	CMCC	*E. coli*		Negative	*E. coli* O157:H7	1	1(100%)
	CMSB	*E. coli*		Positive		1	0(0%)
Total						4	1(25%)

The result obtained above is similar to that of Abong and Momba (2008) who recorded prevalence of *E. coli* O15:H7 ranging from 0% to 33% in onions and cabbage respectively. However, our result is contrary to that of Saeed *et al*. (2013) and Enabulele and Uraih (2009) who did not detect *E. coli* O15:H7 among all the *E. coli* isolated from vegetables. The presence of this pathogen may be due to poor handling as well as inadequate sanitation and hygiene in the market (Dutta *et al*., 2013).

CONCLUSION

Enterohemorrhagic *E. coli* O157:H7 is one of the Shiga-toxin producing types of *E.coli* and one amongst the bacteria associated with various food borne diseases, this have been a threat in both developed and developing countries. Minimally processed and ready to eat leafy vegetables such as cabbage, lettuce, and spinach are nowadays considered as important part of a healthy diet; contamination of these vegetables reflects poor hygienic quality which might be associated with sub-standard farming, handling, packaging and marketing practices.

According to the results of this study *E. coli* O15:H7 is very rare in vegetables, and this warrants further studies most especially focusing on the non shiga-toxin producing type (non-O157 STEC). Improved surveillance programmes are necessary so as to pre-empt any potential outbreaks due to fresh produce which are now increasingly considered as vehicles of infection.

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