African Scientist Vol. 26, No. 3 September 30, 2025
Printed in Nigeria
1595-6881/2025 $80.00 + 0.00

https://africansciientistjournal.org

afs2025004/26301

Prevalence of Gram-Negative Bacteria in Ready to Eat Vegetables Obtained from Farms in Kano State, Nigeria

O.I. Enabulele[1*] M. Dahiru[1,2] and A.F. Eghomwanre[3]

[1]Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City.
[2]Department of Public Health, Cavendish University Uganda, Kampala, Uganda
[3]Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City.

*Corresponding author Email: onaiwu.enabulele@uniben.edu, Tel: +234 (0) 802 339 0936

(Received February 10, 2025; Accepted in revised form July 10, 2025)

ABSTRACT: This study assessed the presence of pathogenic bacteria in ready to eat vegetables from vegetable farms in Kano State, Nigeria. One hundred (100) samples each of carrot, cabbage and lettuce grown in Jakara and Sharada irrigation farms in Kano state were investigated. Samples were cultured and isolates identified by standard bacteriological methods. This study revealed that sixteen different gram-negative bacterial genera, namely, *Escherichia coli, E. vulneris, Shigella* spp., *Tatumella* spp., *Acinetobacter* spp., *Citrobacter* spp., *Enterobacter* spp., *Salmonella* spp., *Hafnia* spp., *Citrobacter* spp., *Klebsiella* spp., *Providencia* spp., *Edwardsiella* spp., *Yersinia* spp., *Proteus* spp. and *Serratia* spp., were isolated from the respective samples. Among the isolated pathogens, *E. coli* (48%), *Citrobacter* spp. (36%) and *Salmonella* spp. (26%) were the most frequently isolated organisms from vegetables at Jakara farms while the predominant organisms isolated from Sharada samples were *Shigella* spp. (18%), *Enterobacter* spp. (32%) and *Salmonella* spp. (34%). Samples were also screened separately for *Campylobacter* spp. and *C. jejuni* was the most common isolate recovered. The presence of these potentially pathogenic organisms poses a serious public health risk to consumers who eat these vegetables in a raw form.

Keywords: Enteropathogens, Health risks, Pathogenic, Prevalence, Vegetables

Introduction

Food-borne diseases encompass illnesses caused by a variety of different agents, including bacteria (*Salmonella* spp., *Campylobacter* spp. *Vibrio* spp. and *E. coli* 0157:H7), preformed bacterial and other biological toxins e.g. paralytic shellfish poisoning, viruses, parasitic organisms and toxic chemicals such as the heavy metals (Collins *et al.*, 2004). The bacterial infections include; diarrhoea, dysentery and typhoid fevers. The burden is high but prevalence of these diseases is difficult to estimate as many cases go unreported. Childhood diarrhoea alone is reported to average 1.7 billion cases yearly and is the third leading cause of death in children (WHO, 2024). Non-typhoidal *Salmonella* diarrhoea alone is reported to cause about 95.1 million cases globally per year and *Shigella* dysentery is estimated at about 11-21 million cases yearly worldwide (Ao *et al.*, 2015).
Common causative agents of diarrhoea include *Salmonella* spp., *Campylobacter jejuni*, Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC) and other diarrhoegenic pathotypes such as *E. coli* O157:H7 which causes endemic bloody diarrhoea that sometimes degenerates to haemolytic uraemic syndrome (a kidney disease). Fruits and vegetables are packed with essential nutrients critical to physical and mental function

(Kaplan, *et al*., 2007). They contain antioxidants such as vitamin C and carotenoids, which play pivotal roles in protecting the body against oxidative stress which can cause and enhance the progression of neurodegenerative diseases, inflammatory diseases, some cancers and some forms of depression (Raison and Miller, 2011).
Worldwide, vegetables such as carrots, cabbage, lettuce, radish and cucumber are eaten raw either individually or made into salads. Vegetables consumed raw are however, being increasingly recognized as important vehicles for the transmission of human pathogens (Ramees *et al.,* 2017). As fresh vegetables are eaten raw or lightly cooked to preserve taste and their nutrient contents, they serve as a potential source of various food-borne infections and outbreaks (Mir *et al*., 2018). Sources of human pathogen contamination in fruits and vegetables at the preharvest stage include soil, irrigation water, inadequately composted animal manure, dust, wild and domestic animals, human handling, water used for pesticide spray, foliar treatments, and growth hormones (Dahiru and Enabulele, 2015; Bhunia, 2018).
Pathogens such as *Escherichia coli* 0157: H7, *Listeria monocytogenes*, and *Salmonella* spp. have been isolated from many fruits and vegetables, including lettuce, cabbage and cucumbers (Zilelidou *et al*., 2015). Numerous studies have revealed that the outbreaks of diseases such as typhoid fever, dysentery, diarrhoea and even cholera were attributed to the consumption of fresh fruits and vegetables (Balali *et al*., 2020). Several outbreaks of *E. coli* 0157:H7 infections have been linked to leafy green vegetable consumption in the United States (FAO, 2020). A significant outbreak of Shiga toxin-producing *E. coli* (STEC) 0157:H7 was also linked to contaminated lettuce in the United Kingdom (CIDRAP, 2022). While there is an increase in the consumption of fresh fruits and vegetables, this increase is greatly threatened by an increase in microbial contamination. There is, however, a paucity of data on outbreaks of gastrointestinal disease from the consumption of fresh vegetables and source tracking of outbreaks due to a lack of proper disease surveillance in Nigeria. In the northern part of Nigeria, outbreaks of diarrhoea disease usually occur in warmer seasons of the year; although *V. cholerae* are usually suspected as etiological agents of these outbreaks, they are never investigated. This study aimed to investigate the prevalence of human entero-pathogens associated with organically grown selected vegetables in farms in Kano State.

Materials and methods

Sources of samples: Samples were collected from farms irrigated with water from Jakara and Sharada canals both in Kano city, northern Nigeria. Samples were collected at three (3) different farms along the canals (initial point of utilization, midpoint and upstream/terminal point of utilization of each canal). One hundred samples each of lettuce (*Lectuca sativa*), cabbage (*Brassica oleracea* var. *capitata*) and carrot (*Daucus carota* subsp. *sativus*) were collected from the irrigation farms at the above-specified points, with each sample being a composite, collected from at least three points on a farm aseptically. The samples were transported in sterile nylon bags to the laboratory for processing.
Bacteriological analysis of samples: One gram (1 g) of each sample was added to 9 ml of trypticase soy broth (TSB) (Lab M, UK), for enrichment for *E. coli* 0157:H7, and buffered peptone water (BPW) at pH 7.5 was used for the isolation of Vibrionaceae while peptone water was used for other Enterobacteriaceae. These were thoroughly shaken and cultured on Plates of Nutrient agar (NA), Eosin methylene blue (EMB) agar and blood agar respectively. The cultures were incubated at 37 ^{o}C for 24 h. The blood agar plates were however incubated in anaerobic jar with CampyGene (Oxoid) incubated at 42 ^{o}C for 24 h for the isolation of *Campylobacter* species. All isolates that had a green metallic sheen on EMB were sub-cultured on Sorbitol MacConkey agar supplemented with cefixime and potassium tellurite (CT-SMAC) and incubated at 37 ^{o}C for 24 h for isolation of *E. coli* O157:H7. Isolates of *E. coli* O157:H7 are non-sorbitol fermenters on CT-SMAC.
All distinct isolates recovered from the agar plates were purified by subculturing, followed by Gram staining and then further identified using Microbact 24E phenotypic identification kit. Tests available on Microbact 24E kit include; fermentation of sugars such as mannitol, xylose, hydrolysis of amino acids such as lysine and ornithine, tryptophan deamination etc. Suspected isolates of *Campylobacter* were further tested for hydrogen sulphide production, hydrolysis of indoxyl acetate and susceptibility to nalidixic acid as well as cephalothin. Suspected *E. coli* O157:H7 isolates were confirmed with *E. coli* O157:H7 latex agglutination kit.

Results

This study revealed the presence of fifteen (15) different gram-negative bacterial genera, namely, *Escherichia* spp., *E. vulneris, Shigella* spp., *Tatumella* spp., *Acinetobacter* spp., *Citrobacter* spp., *Enterobacter* spp.,

Salmonella spp., *Hafnia* spp., *Campylobacter* spp., *Klebsiella* spp., *Providencia* spp., *Edwardsiella* spp., *Yersinia* spp., *Proteus* spp. and *Serratia* spp. in the lettuce, cabbage and carrots respectively (Table 1).
E. coli 0157:H7 was isolated from 21%, 22% and 14% of the lettuce, cabbage and carrot samples, respectively, from the Jakara water-irrigated farms, whereas 10%, 22%, and 10% of the isolates were found in the vegetables from the Sharada farms. The vegetables from both farms also had *Salmonella enterica*, which occurred in 12%, 16% and 34% of the lettuce, cabbage and carrot samples from the Jakara irrigated farms and 20%, 18% and 26% of the positive samples from Sharada. Among the isolated pathogens, *Shigella* spp. were the most prevalent in lettuce (48%) and carrot (24%) at Sharada Farm. Prevalence of *Yersinia* spp. ranged from 0% in cabbage (Sharada) to 20.0% in lettuce (Jakara)
Eight species of the genus *Campylobacter* were also isolated from the samples (Table 2). The results revealed that *C. jejuni* was the most common isolate from lettuce, carrot and cabbage grown at Jakara irrigated farms, with 28%, 28%, and 16% positive samples, respectively. Those grown at the Sharada irrigation sites had 16%, 24% and 4% positive samples, respectively. *C. mucositis* was isolated only from cabbage in Jakara (2%) and Sharada (32.0%). *Campylobacter jejuni* was most predominant with 19.33% prevalence compared to other strains of *Campylobacter* isolated from the vegetables.

Table 1: Prevalence of *Escherichia coli* 0157:H7 and other Enterobacteriaceae strains isolated from lettuce, cabbage, and carrot samples

Bacterial isolates	**Lettuce**		**Cabbage**		**Carrots**		
	No of Isolates (%) in JK (n=50)	**No of Isolates (%) in SH(n=50)**	**No of Isolates (%) in JK (n=50)**	**No of Isolates (%) in SH(n=50)**	**No of Isolates (%) in JK (n=50)**	**No of Isolates (%) in SH(n=50)**	**Total Isolates (%) n=300**
E. coli 0157:H7	1(2.00)	5(10.00)	11(22.00)	11(22.00)	7(14.00)	6(12.00)	41(13.67)
Other E. coli spp.	9(18.00)	8(16.00)	3(6.00)	11(22.00)	3(6.00)	5(10.00)	39(13.00)
E. vulneris	7(14.00)	8(16.00)	0(0.00)	9(18.00)	2(4.00)	11(22.00)	37(12.33)
Shigella spp.	7(14.00)	24(48.00)	2(4.00)	10(20.00)	5(10.00)	12(24.00)	60(20.00)
Tatumella spp.	2(4.00)	0(0.00)	0(0.00)	0(0.00)	4(8.00)	1(2.00)	7(2.33)
Acinetobacter spp.	2(4.00)	0(0.00)	2(4.00)	0(0.00)	16(32.00)	5(10.00)	25(8.33)
Citrobacter spp.	5(10.00)	8(0.00)	21(42.00)	7(14.00)	6(12.00)	12(24.00)	59(19.67)
Enterobacter spp.	9(18.00)	2(4.00)	2(4.00)	16(32.00)	4(8.00)	5(10.00)	38(12.67)
Salmonella spp.	6(12.00)	10(20.00)	8(16.00)	9(18.00)	17(34.00)	13(26.00)	63(21.00)
Hafnia spp.	1(2.00)	6(12.00)	1(2.00)	8(16.00)	4(16.00)	5(10.00)	25(8.33)
Klebsiella spp.	2(4.00)	15(30.00)	9(18.00)	12(24.00)	11(22.00)	13(26.00)	51(17.00)
Providencia spp.	1(2.00)	3(6.00)	3(6.00)	3(6.00)	10(20.00)	2(4.00)	22(7.33)
Edwardsiella spp.	0(0.00)	5(10.00)	0(0.00)	2(4.00)	3(6.00)	2(4.00)	12(4.00)
Yersina spp.	4(8.00)	10(20.00)	0(0.00)	1(2.00)	3(6.00)	3(6.00)	21(7.00)
Proteus spp.	3(6.00)	2(4.00)	5(10.00)	0(0.00)	5(10.00)	7(14.00)	22(7.33)
Serratia spp.	17(32.00)	6(12.00)	13(26.00)	6(12.00)	2(4.00)	7(14.00)	51(17.00)
Morganella spp.	0(0.00)	2(4.00)	0(0.00)	2(4.00)	0(0.00)	3(6.00)	7(2.33)

JK = Jakara canal, SH = Sharada canal

Table 2: Prevalence of *Campylobacter* species isolated from vegetables grown at wastewater irrigation farms

Bacterial Isolates		**Lettuce**		**Cabbage**		**Carrot**	**Total**
	No of Isolates (%) in JK (n=50)	**No of Isolates (%) in SH (n=50)**	**No of Isolates (%) in JK (n=50)**	**No of Isolates (%) in SH (n=50)**	**No of Isolates (%) in JK (n=50)**	**No of Isolates (%) in SH (n=50)**	**No of Isolates (%) n=300**
C. fetus	7(14.00)	7(14.00)	5(10.00)	0(0.00)	9(18.00)	4(8.00)	32(10.67)
C. jejuni/doylei	14(28.00)	8(16.00)	8(16.00)	2(4.00)	14(28.00)	12(24.00)	58(19.33)
C.sputorum biovar bubulus	2(4.00)	6(12.00)	3(6.00)	0(0.00)	3(6.00)	3(6.00)	17(5.67)
C. upsaliensis	2(4.00)	0(0.00)	0(0.00)	3(6.00)	0(0.00)	0(0.00)	5(1.67)
C. coli/jejuni	1(2.00)	5(10.00)	3(6.00)	0(0.00)	3(6.00)	4(8.00)	16(5.33)
C. mucosalis	0(0.00)	0(0.00)	1(2.00)	16(32.00)	0(0.00)	0(0.00)	17(5.67)
C. lardis	0(0.00)	0(0.00)	2(4.00)	0(0.00)	0(0.00)	0(0.00)	2(0.60)
C.sputorum biovar fecalis	0(0.00)	0(0.00)	3(6.00)	0(0.00)	3(6.00)	0(0.00)	6(2.00)
Total	26	26	26	21	32	23	154(51.3)

Discussion

The bacterial isolates obtained from cultured lettuce, cabbage, and carrots from farms in Kano state, Nigeria, included typical enteropathogens such as *E. coli* O157:H7, nontyphoidal *Salmonella* spp., *Shigella* spp., *Yersinia* spp., *Campylobacter jejuni* and others such as; *Acinetobacter and Citrobacter spp*. Earlier studies (Enabulele *et al*., 2008; Odu and Okomuda, 2013; Enabulele and Uraih, 2016; Ssemanda *et al*., 2017; Sane *et al*., 2024) have also documented the presence of these bacteria in these and other vegetables that are normally eaten raw. The presence of these enteropathogens and other coliforms is a serious cause for concern, as it presupposes faecal contamination. These organisms are normal inhabitants of the gastrointestinal tract of many wild/domestic animals as well as men who are either carriers or patients. They find their way into the environment through their faecal waste, from which they can contaminate fresh produce through irrigation water, effluents, improperly composted manure, soil and even bird droppings and flies (Dahiru and Enabulele, 2015).

Humans can become infected through the consumption of those vegetables. Data on outbreaks of infections resulting from the consumption of vegetables are scarce in developing countries, including Nigeria. However, now and then, there are unreported and undocumented reports of outbreaks of diarrhoea and vomiting after eating vegetables/fruit salads at parties. Ohiduzzaman *et al*. (2022) reported that infections caused by *Salmonella spp*., enterohemorrhagic *E. coli* (EHEC) and *Campylobacter jejuni* as a result of the consumption of vegetables occur frequently. In Nigeria, these vegetables are produced mainly in northern states of Nigeria and then transported to other parts of the country where they are sold in open markets and street vending carts. The awareness that vegetables contain numerous phytochemicals, vitamins and fibres that support good health encouraged many people to consume them regularly as part of a healthy diet. Many buy carrots, sliced watermelon, cucumber, along the streets and consume them immediately without bothering to even rinse them. This is worrisome, as they stand the risk of becoming infected. Other isolates, such as *Acinetobacter* spp., *Enterobacter* spp., and *Serratia marcescens* are not typical gastrointestinal tract pathogens. They are part of the normal soil microbiota that can cause spoilage of fresh produce. The survival of faecal indicator bacteria in ambient environments is strongly influenced by abiotic factors such as temperature, salinity, sunlight and biotic factors such as competition and predation (Richard *et al*., 2004); however, it has been reported that these bacteria can survive on lettuce for up to 14 days if these and other vegetables are eaten raw, they should be thoroughly washed under running portable water (Luna-Guevara *et al*., 2019).

Conclusion

Enterohaemorrhagic *E. coli* 0157:H7 and other enteropathogenes such as *Vibrio* spp, *Campylobacter* spp, *Salmonella* spp. and *Shigella* spp. were detected in cabbage, lettuce and carrot samples collected from vegetable farms in Kano state, Northern Nigeria. The presence of these potentially pathogenic organisms is a public health risk when the vegetables are consumed. The triple-wash method is recommended to minimize the possibility of contracting infections such as diarrheoa, typhoid fever, dysentery and even cholera. It is recommended that Government authorities should also put in place and enforce guidelines for the use of wastewater for irrigation purposes. Farmers should also be encouraged to use composted manure so as to reduce the level of potential contamination of fresh produce with pathogenic microorganisms

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