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Occurrence of Enteric Pathogens on Fresh Produce Grown on Irrigated Soils

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To assess the potential health risks of fresh produce grown on irrigated soils treated with manure in Kano State, a large produce region in Nigeria.
Methodology: Fresh produce irrigated with wastewater on manure treated soils were assessed for the prevalence, serotype distribution and toxigenicity of *Escherichia coli* O157, *Salmonella* spp and *Vibrio cholerae* in a large produce region in Nigeria. A total of 230 samples obtained from five designated produce locations were examined using selective isolation method with prior enrichment. Fresh produce comprised carrots, cabbage, cucumber, lettuce, tomatoes, spinach, green peas and spring onions. Suspect isolates were identified and characterized by conventional biochemical methods and Microbact 24E (Oxoid, UK) kit. Confirmed isolates were serotyped and *E. coli* O157 and *Vibrio cholerae* O1 were assayed for their toxigenic potentials using the Reverse Passive Latex Agglutination kit. The enterotoxigenicity of *Salmonella* spp was determined by detection of *stn* gene using polymerase chain reaction (PCR) techniques.

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Results: Results obtained showed that overall, *Salmonella* spp and *Escherichia coli* O157 had the highest prevalence of 17.0% and 10.9%, respectively. Both were most commonly detected from fresh produce. The serotypes of *Salmonella* detected include *Salmonella typhi* (51.3%), *Salmonella paratyhpi* (20.5%) and *Salmonella typhimurium* (28.2%); strains of *Vibrio cholerae* O1 detected include *Vibrio cholerae* O1 of the Ogawa, Inaba and the Hikojima serotypes.
Conclusion: The use of untreated irrigation water in vegetable production represents a significant route of transmission of diarrheal pathogen to humans and hence represents a public health risk. We recommend proper and adequate wastewater treatment before use.

Keywords: Enteric pathogens; fresh produce; contamination; irrigation water.

1. INTRODUCTION

Developing countries are disproportionally affected by lack of access to clean water, hence the re-use of wastewater for crop irrigation offers a very promising water conservation alternative [1]. Secondly, indiscriminate disposal of untreated wastes, which are often heavily laden with pathogenic microorganisms into aquatic environments near cities, could serve as potential dangers to human health [2]. Wastewater reuse in developing countries is shown to be responsible for approximately 4 billion cases of diarrhea that cause about 2.2 million deaths a year [3]. Although 17-25% of the costs from illness in Nigeria are spent on foodborne illness, foodborne pathogens still accounts for about 200,000 deaths [4]. Pathogens persist in the environment or in multiple hosts and contaminate the foods through pathways that reflect the variety of ecosystems that make up our food supply [5]. Most of the pathogens identified as causative agents in these illnesses or outbreaks are enteric zoonotic pathogens that are typically associated with animal hosts [6]. *Escherichia coli* O157, *Salmonella* spp and *Vibrio cholerae* are major food borne pathogens [7] and their implication in documented illness associated with consumption of vegetables has increased in recent years [8]. Studies have demonstrated a very close relationship between the consumption of fresh produce irrigated with raw wastewater to many food borne diseases like gastroenteritis and cholera [9]. From a previous study, [10] assessed the prevalence of *Escherichia coli* O157 in diarrheal patients and surface water in Zaria, Nigeria and found 2.2% in surface waters and 5.4% in children with diarrhea. While, [11] reported a 33% incidence of pathogens on irrigated vegetables and irrigation water in Zaria, Nigeria. In 2010, there was marked severe outbreak of gastroenteritis caused by *Vibrio cholerae*, which started from the northern part of Nigeria, spreading to the other parts and involving approximately 3,000 cases and 781 deaths [1] and Kano State was the most affected, the State is particularly affected by cholera outbreaks [12]. Northern Nigeria has a markedly long dry season, hence irrigation is needed to provide water for year round agricultural production. In Kano State, untreated industrial, domestic and abattoir wastewaters are discharged into streams and rivers which are used for irrigation by farmers [13]. This work therefore, assessed the potential health risks of fresh produce grown on irrigated soils treated with manure in Kano State, a large produce region in Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

Kano State, Nigeria covers an area extending between latitudes 12º40′ and 10º30′ and longitude 7º40′ and 9º30′ [14]. It is the largest and most populated State in northern Nigeria and the largest in population in the country after Lagos [15]. Its population could be attributed to its large number of industries, tanneries, textiles and other allied companies which makes it a high commercial State. Farming of crops such as tomatoes, spinach, green pepper, lettuce, cabbage and other fresh produce in close proximity to livestock production is a common practice of the people.

2.2 Sample Collection

Stratified simple random sampling was carried out to select five major irrigation sites in the State. Stratification was based on availability of fresh produce such as carrots, cabbage, lettuce, cucumber, spinach, green peas, green beans and spring onions on the farms and the cooperation of the farmers and sources of point source contaminants to water used to irrigate the fresh produce. All sites had similar crop structures. Sample collection was carried out on

a weekly basis from all the study sites from May, 2010 to March, 2011. Sources of contamination to the sites include abattoir wastes channeled from a large abattoir at site 1, a refuse dump adjacent to site 2, domestic sewage and waste from nearby mechanic workshops were channeled to site 3, industrial waste channeled to the river used for irrigation by farmers at site 4. Site 5, the Kadawa, Ahmadu Bello University irrigation station at the Tiga dam, served as control since irrigation water underwent some form of treatment (waste stabilization pond) before use.

A total of 230 (94 fresh produce, 57 irrigation water and 79 soil/manure) samples, which consisted of; 62 samples from site 1, 72 from site 2, 60 from site 3, 14 from site 4 and 22 samples from site 5 were collected and screened for *Escherichia coli* O157:H_7, *Salmonella* and *Vibrio cholerae*. Fewer samples were collected from site 4 due to lower availability of produce at time of sampling and cooperation of fewer farmers. Water samples were collected according to the procedure recommended by American Public Health Association [16] in sterile wide mouth, screw capped 250 ml bottles. Representative soil/manure samples were collected aseptically using ethanol-sterilized spatula. About 100 g of sample was collected at designated site at five different points, one from the center and at four different points, on the periphery and mixed together. Fresh produce available in the study sites during the study period were purchased directly from the farmers on the farm. Produce and environmental samples were obtained at the same time from the various study sites during sample collection. Using 90% ethanol sterilized scissors; the fresh produce was cut into factory sterile polythene bags. All samples were packed on ice during transportation to the laboratory for analysis. Samples were analyzed within six hours of collection.

2.3 Enrichment and Isolation

Isolation and identification of *E. coli* O157:H_7 was obtained from each sample using enrichment in *Escherichia coli* (EC) medium and plating on Cefixime tellurite sorbitol MacConkey (CT-Smac) agar method [17]. Briefly, 20 ml of each water sample was combined with equal volume of sterile EC medium (Oxoid) in a cotton plugged Erlenmeyer flask and incubated at 37ºC for 24 h. Twenty five grams of cut vegetables was enriched in 225 ml buffered peptone water (oxoid) and incubated at 37ºC for 8 h [17]. Five millilitres of the broth was enriched in equal volume of EC medium and incubated at 37ºC for 24 h. One gram of well mixed soil or manure was placed in 9 ml of EC medium and incubated at 37ºC for 24 h for enrichment. Enriched broth from each sample was then streaked on sorbitol MaConkey agar (Oxoid) plates containing cefixime (0.5 mg/liter) and potassium tellurite (2.5 mg/liter) and incubated at 37ºC for 24 h [17]. Three to four sorbitol negative colonies exhibiting *E. coli* O157:H_7 colony phenotype (gray or pale with a darker center) were selected as presumptive *E. coli* O157:H_7 isolates, inoculated into slants of nutrient agar, incubated at 37ºC for 24 h and stored in the refrigerator at 4ºC as stock cultures for further tests.

Salmonella was isolated from samples using pre-enrichment in peptone water, then enrichment in selenite F broth followed by isolation on *Salmonella –Shigella* agar [3]. Ten millilitres of each water sample was inoculated in ten millilitres peptone water, incubated at 37ºC for 24 h. One millilitre of the broth was transferred into 9 ml selenite F broth for selective enrichment and incubated at 37ºC for 24 h. Twenty five (25 gm) of cut, washed vegetables was pre-enriched in 225 ml sterile peptone water and incubated at 37ºC for 24 h. One millilitre of the broth was transferred into 9 ml selenite F broth for selective enrichment [11]. Twenty five (25 gm) of well mixed manure treated soil was pre-enriched in 225 ml sterile peptone water and incubated at 37ºC for 24 h. One millilitre of the broth was transferred in 9 ml of selenite F broth and incubated at 37ºC for 24 h. After the enrichment, *Salmonella* was detected by plating the broth cultures on *Salmonella-Shigella* (SS) agar and incubation at 37ºC for 24 h. Typical *Salmonella* colonies which appeared colorless with black centers were picked and confirmed as Gram negative by Gram-staining. Pure cultures of isolates were made on slants of nutrient agar, incubated at 37ºC for 24 h and stored in refrigerator at 4ºC as stock cultures of presumptive *Salmonella* for further identification.

Vibrio was isolated from samples using the method of [18]. Samples were pre-enriched in alkaline peptone and incubated at 37ºC for 24 h. Ten millilitres of each water sample was inoculated in ten millilitres peptone water, incubated at 37ºC for 24 h. One millilitre of the broth was transferred into 9ml alkaline peptone water for selective enrichment and incubated at 37ºC for 24 h. Twenty five (25 gm) of cut, washed vegetables was pre-enriched in 225 ml

sterile peptone water and incubated at 37ºC for 24 h. One millilitre of the broth was transferred into alkaline peptone water for selective enrichment [11]. Twenty five (25 gm) of well mixed manure treated soil was pre-enriched in 225 ml sterile peptone water and incubated at 37ºC for 24 h. One millilitre of the broth was transferred in 9ml of alkaline peptone water and incubated at 37ºC for 24 h. The enriched broth was streaked on thiosulphate citrate bile salt (TCBS) agar and incubated at 37ºC for 24 h. Mixed cultures were transferred to fresh (TCBS) agar plates to obtain pure cultures. Typical *Vibrio* colonies, which appeared yellow, were picked and confirmed to be gram negative. Pure isolates were stored on nutrient agar slants as stock cultures of presumptive *Vibrio* isolates for further tests.

2.4 Biochemical Characterisation of Isolates

Biochemical characterization tests including indole, methyl red, citrate, Voges-Proskauer, motility, oxidase, hydrogen sulphide production and reaction on triple sugar iron tests were based on standard techniques [19]. Microbact (Oxoid, UK) 24E Gram-negative bacillus (GNB), a standardized micro-substrate system designed to simulate conventional biochemical substrates for the rapid identification of *Enterobacteriaceae* was further used to confirm isolates previously identified by the conventional biochemical characterization. A 24h culture of the presumptive isolates on selective media was obtained and the test was carried out and interpreted as recommended by the manufacturer. An 8 digit code was then obtained which was fed into the computer identification software which immediately gave the probable identity of the organism tested in percentage. The microbact software recommends a 75% cut-off point for a probable identification. Tests that gave less than 75% were not accepted for the bacteria targeted.

2.5 Serological Identification of Isolates

All identified isolates were further confirmed serologically by slide agglutination. Sorbitol negative *E. coli* isolates were tested using *E. coli* O157 latex test kit (Oxoid, Basingstoke, and Hampshire, England). A further determination of the O- antigen and H7 flagella antigen was carried out using specific antiserum (Denken Seiken, Japan) by the slide agglutination method. *Escherichia coli* ATCC 25922 was used as a negative control. *Salmonella* serotyping was done according to Kauffman-White Scheme [20] involving the use of *Salmonella* antiserum. Polyvalent *Salmonella* antiserum A-G, A-S, surface antigen vi, phase1 and phase 2 flagella H antigens (Denka Seiken, Japan) were used for the serological identification of *Salmonella*. Serological identification of *Vibrio cholerae* was carried out with the use of *Vibrio cholerae* antiserum (Denka Seiken, Japan) consisting of polyvalent antiserum (containing antigenic factor A, B and C), *V. cholerae* serovar Ogawa antiserum (factor B), *V. cholerae* serovar Inaba antiserum (factor C) and the *V. cholerae* O139 ``Bengal`` antiserum.

2.6 Detection of Toxin from Isolates of *E. coli* O157:H7 and *V. cholerae* Obtained in the Study

The reverse passive latex agglutination test (RPLA) method was used for the detection of VT1 and VT2 from *Escherichia coli* O157:H_7 and cholera enterotoxin from isolates of *Vibrio cholerae* O1. Toxin production and extraction was performed using the solid culture method. The isolated organism was inoculated onto brain heart infusion agar (Oxoid) slopes and incubated at 37ºC for 30 minutes for extraction, with occasional shaking. After extraction, the culture was centrifuged at 4,000 rpm for 20 minutes at 4ºC. The filtrate was retained for the verocytotoxin assay. The assay was carried out according to kit manufacturer`s instruction (Oxoid). Toxin controls (TD0964 and TD0965) provided reference for the positive patterns. To a row of microtitre plates arranged in three columns each consisting of 8 wells, 25 µl of dilluent (TD0966) was dispensed into each well using a micropipette. Twenty five (25 µl) of test sample supernatant was added to the first well of each column, a microdiluter was used to pick up 25 µl of the resultant suspension and doubling dilutions was carried out down each column, up to and including row 7.25 µl of sample and buffer mix was removed from the 7^{th} well and discarded. With the last row of well left containing diluent (TD0966) only, 25µl of the test latex VT1 (TD0961) was added to each well in the first column. About 25 µl of test latex VT2 (TD0962) to each well in the second column and 25 µl of latex control (TD0963) was added to each well in the third column to detect false agglutination reactions. The toxin controls (TD0964 and TD0965) were also assayed in the same manner as test samples. The microtitre plates were incubated in a moisture box in a vibration free

surface at room temperature for 24h. Each well in each column was examined for agglutination against a black background after 24 h. Agglutination patterns were recorded as negative (−, ±) and positive (+, ++ and +++). For the *Vibrio cholerae* toxin assay, isolates of *Vibrio cholerae* were inoculated in alkaline peptone water adjusted to pH 8.4 and incubated at 37ºC for 24 h. Two (2 ml) mililiters of the broth was centrifuged at 3,000 rpm for 20 minutes at 4ºC. The supernatant obtained served as the test sample. Twenty five microlitre of dilluent was dispensed in two rows of microtitre plates each consisting of eight wells except the first well in each row. To the first and second wells of each row 25 µl of test sample was added. Starting at the second well of each row, doubling dilution was carried out along each of the two rows except the last well (leaving the last well containing diluent only). Twenty five (25 µl) of sensitized latex was added to each well of the first row, while 25 µl of latex control was added to each well of the second row. The contents of each well were mixed together on agitation. The microtitre plates were then placed in a moisture box and left undisturbed on vibration free surface at room temperature for 24h. Agglutination patterns were read as (+), (++) and (+++) and considered positive while non agglutination was considered negative (Oxoid, Basington, England).

2.7 Polymerase Chain Reaction

All confirmed *Salmonella* enterica isolates were subjected to Polymerase Chain Reaction (PCR), with the *Salmonella* enterotoxin *stn* gene being the targeted gene. Isolates were inoculated into 10ml of Luria Betani (LB) medium and incubated for 24h at 37ºC. DNA extraction was carried out using the ZR fungal/bacterial DNA mini prep™ (Zymo Research, Invine, CA, USA). All protocols were followed and ultra-pure DNA was eluted into 50 µl DNA elution buffer.

2.8 Primers

Primers used were obtained from Fermentas™, Germany and were designed based on the sequence of the *stn* gene [21]. Forward primer 5`-TTGTGTCGCTATCACTGGCAACC-3` and reverse primer 5`-ATTCGTAACCCGCTCTCGTCC-3` with an expected amplicon size of 284 bp. PCR was carried out in a total volume of 25 µl containing 3 µl template DNA, 0.5 µl of the forward primer, 0.5 µl of reverse primer, 12.5 µl Dream Taq PCR mastermix containing dNTP`s, taq polymerase and 4 mM $MgCl_2$, 8.5 µl of nuclease free water was also added. PCR was performed in a DNA thermal cycler (Applied Biosystems, Gene Amp PCR systems 9700). After initial denaturation step of 5min at 94ºC, 35 cycles of amplification were performed. Each cycle consisted of the following steps; 1 min at 94ºC (denaturation), 90 seconds at 60ºC (Primer annealing) and 1 min at 72ºC (extension) and 72ºC for 10min for final extension. Ten microlitres of the reaction mixture was mixed with gel loading buffer and then resolved by electrophoresis on 2% agarose gels with the 100 bp DNA ladder (Fermentas™, Germany). The reaction products were visualized by staining with ethidium bromide. Image documentation was carried out with a Gene snap ultraviolet transilluminator machine (Applied Biosystems) and viewed on a computer.

3. RESULTS

E. coli O157:H_7 and *Salmonella* spp. were mostly frequently isolated from fresh produce with isolation rates of 19.1% and 20.2% respectively, while, *Vibrio cholerae* was most isolated from soil/manure samples with an isolation rate of 18.9% (Table 1). Higher numbers of targeted pathogens were obtained from sites 1, 2 and 3 as compared to pathogen recovery from sites 4 and 5. Pathogens were most abundant in samples from site 1 (Table 2). Serological characterisation of three target *Salmonella* serotypes obtained in the study showed; *Salmonella enterica* subsp *typhi*; 20(51.3%), *Salmonella enterica* subsp *paratyphi*; 8(20.5%) and *Salmonella enterica* subsp *typhimurium;* 11(28.2%) (Table 3). *S. enterica* subsp *typhi* isolates were mostly detected in fresh produce. Serological identification confirmed and showed the distribution of 28 *Vibrio cholerae* serotypes of which 16 were *Vibrio cholerae* O1 consisting of 3(1.3%) Inaba, 6(2.6%) Ogawa and 7(3.0%) Hikojima, while 12(5.2%) were non- O1 *V. cholerae* (Table 4).

Of the 14 representative isolates of *E. coli* O157:H_7 (ten from fresh produce, two from irrigation water and two from soil/manure) assayed for verocytotoxin (VT1 and VT2) production, four (28.6%) produced VT1, one (7.1%) produced VT2 and seven (50.0%) produced both VT1 and VT2 Of the 4 isolates that produced VT1, two were isolates from fresh produce, one from irrigation water and one from soil/manure samples. Out of the seven isolates

that produced both VT1 and VT2; five were isolates from fresh produce, two from irrigation water samples and one from soil/manure samples (Table 5). The *Vibrio cholerae* isolates were tested for toxin production consisting of five from fresh produce, four from irrigation water samples and four from soil/manure samples, 8(61.5%) produced cholera toxin, of which three were isolates from fresh produce, three from water and two from soil/manure samples (Table 6).

Virulence potential of representative some representative *Salmonella* serotypes by PCR showed that all S*almonella* isolates gave a 284 bp DNA fragment of the s*tn* gene confirming their virulence potentials (Fig. 1).

Table 1. Frequency of detection of enteric pathogens in fresh produce, irrigation water and soil/manure samples

Sample type	No. tested	No. (%) contaminated with enteric agents		*Vibrio cholerae*
		***E. coli* O157:H7**	***Salmonella* spp**	
Fresh produce	94	18(19.1)	19(20.2)	3(3.2)
Irrigation water	57	7(12.3)	11(19.3)	10(17.5)
Soil/Manure	79	3(3.8)	9(11.4)	15(18.9)
Total	230	25(10.9)	39(17.0)	28(12.2)

key---- No= Number tested, (%) = percent

Table 2. Contamination of vegetables, irrigation water and soil/manure samples obtained from different sites with enteric pathogens

Sampling sites no tested	Sample types	No	Pathogens detected			Total pathogens present (%)
			***Escherichia coli* O157:H_7**	***Salmonella* spp**	***Vibrio cholerae***	
Site 1(62)	Fresh produce	22	7	11	2	20 (90.9)
	Water	20	2	6	3	11 (55.0)
	Soil/Manure	21	1	2	4	7 (33.3)
Site 2 (72)	Fresh produce	31	5	3	2	10 (32.3)
	Water	20	2	3	2	7 (35.0)
	Soil/Manure	21	-	1	7	8 (38.1)
Site 3(60)	Fresh produce	23	3	5	-	8(34.8)
	Water	15	3	4	2	9(60.0)
	Soil/Manure	22	-	2	2	4 (18.2)
Site 4 (14)	Fresh produce	7	1	1	-	0(0.0)
	Water	3	1	1	1	1 (0.25)
	Soil/Manure	4	-	-	2	1 (33.3)
Site 5 (22)	Fresh produce	25	-	-	-	0 (0.0)
	Water	4	-	1	-	1 (0.25)
	Soil/Manure	3	-	-	1	1 (33.3)

Key; site 1=abattoir liquid waste, site 2=domestic sewage drain, site 3=human sewage/industrial waste, site 4= industrial effluents and site5=control, No = Number Tested

Table 3. Distribution of *Salmonella* serotypes in fresh produce, irrigation water and soil/manure samples

Sample type	No tested	*S. typhi*	*S. paratyphi*	*S. typhimurium*
Fresh produce	19	10	2	7
Soil/manure	11	5	3	2
Water	9	5	3	2
Total	39	20(51.3%)	8(20.5%)	11(28.2%)

Table 4. Distribution of *Vibrio cholerae* O1, O139 and non O1 *V. cholerae* in fresh produce, irrigation water and soil/manure samples

Sample type	No. of samples tested	No of *Vibrio cholerae* O1			Non O1 *Vibrio cholerae*
		Inaba	Ogawa	Hikojima	
Fresh produce	94	-	2	-	1
Water	57	2	3	-	5
Soil/Manure	79	1	1	7	6
Total	230	3 (1.3%)	6 (2.6%)	7 (3.0%)	12 (5.2%)

Table 5. Detection of verocytotoxins (VT1, VT2) from *E. coli* O157:H7 isolates

Sample type	No. of samples analysed	VT1 No (%)	VT2 No (%)	VT1 /VT2 No (%)
Fresh produce	10	2(14.3)	1(10.0)	5(71.4)
Water	2	1(7.1)	0(0.0)	2(28.6)
Soil/manure	2	1(7.1)	0(0.0)	1(14.3)
Total	14	4(28.6)	1(7.1)	7(50.0)

Key: Vt1= verocytotoxin 1, Vt2= verocytotoxin 2, No (%) = Number (percent)

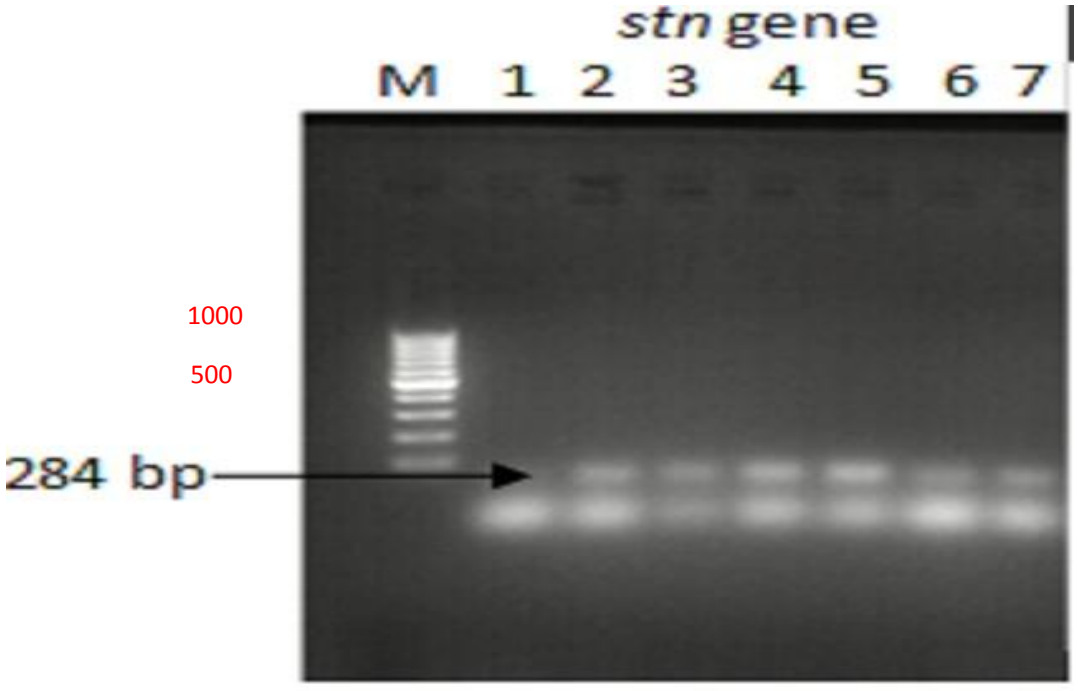

Fig. 1. PCR amplification of the enterotoxin (*stn* gene) in *Salmonella* isolates. Lane 1-7 represents *Salmonella* isolates, M represent molecular weight marker, 1K bp ladder Fermentas™ , Germany). (1-7= Kvb1, Kvb27, Kvb2, Ksb33, Kma33, Kwb34, Kwb23, K=Kano, v=vegetables, m=manure, s=soil, w=irrigation water)

Table 6. Detection of cholera toxin (CT) from *Vibrio* isolates

Sample type	No of isolates analysed	No positive for cholera toxin
Fresh produce	5	3
Water	4	3
Soil/manure	4	2
Total	13	8(61.5%)

4. DISCUSSION

Fresh produce are now recognized as major vehicles of food-borne disease outbreaks and the primary sources of contamination of this commodity group are animal manure amended soils and use of untreated irrigation water [6]. Our study revealed the presence of enteric pathogens; *Escherichia coli* O157:H_7, *Salmonella* spp and *Vibrio cholerae* with isolation rates of 10.9%, 17.0% and 12.2% respectively. Distribution of targeted pathogens showed that *E. coli* O157:H_7 and *Salmonella* spp were more frequently recovered from fresh produce in the study. The higher detection of these pathogens from produce is not unrelated to the large surface area of these vegetables exposed to contact with the effluent/point source contaminants from irrigation water and the type of irrigation system used. Farmers in the study area employ the surface irrigation system. Surface irrigation has been reported to lead to higher contamination of fresh produce as the effluents are discharged directly on the crops [22]. Fresh produce analyzed include tomatoes, cabbage, lettuce, green pepper, onions, amaranth, carrot and cucumber which are commonly eaten uncooked. The concern here is heightened by the increasing demand for fresh produce and increased focus on raw vegetables as a healthy food source [23] and more recent reports of the implication of fresh produce in foodborne illnesses [23]. *Salmonella* spp had the highest isolation rate in the study and this supports previous reports that *Salmonella* can exist in the farm environment due to circulation within the farm and between different pools such as animals, excrement, soil and plants [23].

Reported outbreaks of foodborne illnesses related to produce have been linked to irrigated produce with water contaminated by animal faeces, discharges from sewage treatment facilities or surface run-off [24]. Accordingly, more pathogens were isolated from samples obtained from site 1, where water used in irrigation is strongly influenced by animal discharges from an abattoir situated about 1km from it. Specifically, higher number of *E. coli* O157:H_7 was obtained from this site. Available information indicates that the carriage of *E. coli* O157 in cattle was an important factor in the emergence of this pathogen in Africa [25]. The prevalence of 10.9% of confirmed *E. coli* O157:H_7 in this study is higher than the report of [10] which detected 2.1% of this organism from river used for fresh produce irrigation in Zaria, Nigeria. *Vibrio cholerae* was observed to be highest (12.2%) in soil/manure in the study. This represents a public health risk, as manure is used as fertilizer by the farmers without prior treatment. The increased use of organic sources for plant nutrients, as dictated for organic farming practice, will unavoidably lead to increased exposure [23] and transmission to man. The 18.9% overall prevalence of *Vibrio cholerae* obtained is higher than the 0.59% isolation rate reported from manure obtained from households engaged in livestock farming in Zaria, Nigeria [26]. Sites1, 2 and 3 which received effluents from abattoir, domestic and human waste showed higher presence of the organisms than sites 4 and 5. The higher occurrence of these pathogens in the former could be due to higher anthropogenic activities than in the latter. Previous studies showed that industrial effluents discharge may cause impaired movements, behavioral and physiological responses, histological and reproductive abnormalities [27]. Irrigation water at site 4 which receives influx of industrial waste effluents; industrial waste could contain some inorganic constituents which could be deleterious to microbial survival. Lower distribution of pathogens in samples from site5 could be attributable to the waste stabilization pond available at the irrigation station, in which water is held for a period before release for irrigation. However, the detection of some pathogens from samples obtained from this site which served as control in the study supports earlier reports that even soil irrigated with non-faecally polluted tap water yielded pathogens [28]. Thus, it is likely that the pathogens isolated from some samples obtained from this site originated primarily from the external environment rather than the irrigation water [28].

Recent outbreaks of disease caused by *Salmonella* contaminated water and produce prove that *Salmonella* strains in environmental sources influence human health [29]. The distribution of *S. typhi*, *S. paratyphi* and *S.*

typhimurium in the samples examined gives credence to reported implications of produce in foodborne illnesses [29]. The enterotoxin gene was detected in all the *Salmonella* species isolated in the study. This gene is essential for virulence and alters fluid and electrolyte secretion in the intestine [30], hence risks of gastroenteritis on consumption of improperly cooked fresh produce. Similar strains of *V. cholerae* obtained in this study have been reported to have caused epidemics and cholera outbreaks [31]. Strains assayed were toxigenic, hence they pose a great risk to public health not only to the inhabitants in the study area but to the entire country. The 2010 cholera outbreak was reported to be due to hyper- virulent strains of the organism [32] which was reported to have spread widely. Hyper virulent strains in the reported outbreak were observed to have spread sporadically.

5. CONCLUSION

Diarrhea causing agents were isolated from fresh produce and environmental samples analyzed in this study. Enteric pathogens confirmed in this study have been implicated in several cases of diarrhea and foodborne outbreaks. The public health implication of the use of untreated wastewater to irrigate fresh produce is established. Due to the problem of water shortage in Nigeria as in many other developing countries, wastewater reuse in agriculture is almost inevitable, therefore we advocate pretreatment before use. The release of effluent discharge into rivers and streams have great implications on the health of the populace, hence, laws should be enforced to ensure adequate treatment before its discharge.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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