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Occurrence and molecular characterization of salmonella in ready-to-eat salads sold in Ado-Ekiti, Nigeria

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Abstract

Vegetable salads are main dish of daily meals served in homes, schools and restaurants. However, it has been found to be associated with the outbreaks of foodborne diseases in many countries around the world. This study investigated the microbiological quality of vegetable salads sold in Ado-Ekiti, Nigeria, particularly the presence of *Salmonella* spp. Samples examined included different types of fresh ready-to-eat (RTE) vegetable salads. A total of 170 vegetable salad samples were collected from fast food outlets and street vendors. Microbiological examinations included total viable count and total coliform count using pour plate technique, and isolation of *Salmonella* spp. using *Salmonella-Shigella* agar. DNA extraction, PCR and 16S rRNA sequencing were performed to determine the *Salmonella* spp. isolated. A total of 8 (4.7%) and 9 (5.2%) of the salads were below standard guidelines for total viable count and total coliform count respectively. *Salmonella* was isolated from 66 (38.8%) samples. There was no statistical significant correlation (P >0.05) between all sample sources and the different types. *Salmonella* spp. was resistant to cephalosporins and β-lactamase inhibitors, producing five different antibiotic resistance profiles. Representative isolates were homologous to sequences on NCBI database revealing three subspecies of *Salmonella enterica*. This survey showed that RTE vegetable salads consumed from fast food outlets and street vendors in Ado Ekiti, Nigeria are of public health concern, hence the need for government health agencies to monitor and regulate their production.

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Introduction

Food contamination is generally defined as foods that are spoiled or tainted because they either contain microorganisms such as bacteria, parasites or toxic substances that make them unfit for consumption. A food contaminant can be biological, chemical or physical in nature (Malik, 2016). Common foods associated with food contamination are eggs, poultry, meats, unpasteurized milk or other fluids, cheese, raw fruits and vegetables, nuts and spices (Sowjanya, 2016). Freshly consumed vegetables especially those used in salad mixtures, have been implicated in food poisoning and are therefore hazardous to the health of the consumers. This could be linked to the fact that most of these vegetables are consumed without being subjected to thermal process or even thorough washing (Aboh *et al.,* 2011).

A salad is a dish consisting of a mixture of small pieces of food, usually vegetables. However, different varieties of salads may contain virtually any type of ready-to-eat food. Salads are typically served at room temperature or chilled, with notable exception of south German potato salad which is served warm (Avazpour *et al*., 2013). Green salads use a variety of leafy greens, they are common enough that the word salad alone often refers specifically to green salads. Salad may be served at any point during a meal. Appetizer salads which are light, smaller portion salads are served as the first course of a meal. Side salads are served to accompany the main course as a side dish (Joana *et al*., 2011). In Nigeria, vegetable salad is a very common food accompaniment usually made up of tomatoes, cucumber, carrots, green pepper, cabbage and lettuce and sold in almost every market and hawked around by traders. These vegetables can become contaminated during growth, harvesting, processing, storage and shipping. During food preparation in a restaurants or homes, if food preparers do not thoroughly wash their hands, kitchen utensils, cutting boards, and other kitchen surfaces that come in contact with the raw foods. Examples of bacteria that cause foodborne disease include *Salmonella, Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, Vibrio cholerae and Clostridium botulinum.* Viruses like Norovirus, Hepatitis A and parasites like *Cryptosporidium parvum* and *Giardia intestinalis* (Scallan *et al.,* 2011). The manure used to promote the growth of crops and vegetables contain a large number of pathogenic microorganisms (Farjana and Rashed, 2012).

Salmonellosis which is an infection caused by *Salmonella* is of public health concern because most of the strains of *Salmonella* are potentially pathogenic to humans and animals. It also causes morbidity and in some causes mortality having the potential for foodborne zoonotic transmission (Lutful, 2010), affecting both humans and animals worldwide and Nigeria is not an exception. Although most of the infections in humans cause mild gastroenteritis, life-threatening systemic infections are common especially among high risk categories such as infants, children, elderly and immuno compromised individuals (Idowu *et al*., 2017).

Studies have showed that individuals in Ado–Ekiti, Ekiti State Nigeria are at risks of *Salmonella* infection (David *et al.,* 2015). Ado-Ekiti, the State capital of Ekiti State, is situated entirely within the tropics with a total land area of about 5,887.890km^2 (Kayode *et al*, 2016), located between longitudes 40 33' and 50 55' East of the Greenwich meridian and latitudes 70 15' and 80 5' North of the Equator (Arowosegbe *et al.,* 2018). The State enjoys a tropical climate with two distinct seasons; the rainy season (April - October) and the dry season (November - March), with temperature ranges between 21^{o}C and 38^{o}C. The population of people in Ekiti state is estimated at 3,480,122, while the population within Ado-Ekiti is estimated at 512,263 (City population worldwide, 2018).

This study therefore sought to investigate the microbial quantity and quality of RTE salads sold within the metropolis of Ado-Ekiti.

Materials and methods

Sample Collection

One hundred and seventy samples were obtained: 85 fresh vegetable salads from ready-to-eat outlets (fast-food cafeteria) in Ado Ekiti, 85 fresh vegetable salads from street vendors in Ado Ekiti and 30 clinical isolates of *Salmonella* obtained from Ekiti State University Teaching Hospital Ado Ekiti. The vegetable salads were bought in their RTE state between 9am and 10am daily from October 2018 to December 2018 and transported to the laboratory on ice packs within 2 hours after purchase for bacteriological analysis. The salad type was designated into three categories; single vegetables for salad without dressing and non-vegetable ingredients, salad with dressing for salad which contains dressing without non-vegetable ingredients while mixed vegetables was for salad with dressing and non-vegetable ingredients.

Preparation of Sample Homogenate

Twenty five grams (25g) of each vegetable salad sample was homogenized with 225mL peptone water and serial dilutions were prepared up to 10^{-5} following the standard methods for plating (Farahnaaz *et al*., 2013). Using the streak plate technique, 1ml each of 10^{-3} and 10^{-5} diluents were inoculated on S*almonella Shigella* agar while using pour plate technique on nutrient agar and MacConkey agar (Angela *et al*., 2010).

Microbiological investigation and detection of Salmonella spp

The investigation included different microbiological parameters such as Total Viable Count (TVC) and Total Coliform Count (TCC) while *Salmonella* spp. was used as an indicator organism. The standard published by the Food Standards Australia New Zealand, 2010 was employed to determine the safety status of samples.

Antimicrobial Susceptibility Test

This was carried out according to the method of Coorevits *et al*. (2015). Using antimicrobial disc placed on surface of Mueller-Hinton agar plate inoculated with test isolates.

DNA extraction and Purification

Isolates used were first cultured in broth overnight. This procedure was carried out using the Zymo BIOMICS™ DNA Miniprep Kit (CA, USA). Procedure was carried according to manufacturer's instruction. DNA concentration was quantified using a NanoDrop 1000 Spectrophotometer (Nano Drop Technologies, Wilmington, DE, United States). The A260/A280 absorbance ratio was used to determine undesired contaminations. To evaluate the quality and intactness of the extracted DNA, gel electrophoresis was used. The extracted DNA (5µl) was loaded on 1.5% agarose gel (Invitrogen, California, United States), which contained ethidium bromide (1µg/ml) for DNA staining. For image acquisitions, a G:Box™ gel documentation system (Syngene, Cambridge, United Kingdom).

Polymerase Chain Reaction

The PCR was done according to the method of Reza *et al*. (2012) using 16SF (GTGCCAGCAGCCGC GCTAA) and 16SR (AGACCCGGGAACGTA TTCAC) primers. PCR for the amplification of the 16S rRNA procedure was at 94°C for 5mins for initial denaturation, followed by 36 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds and elongation at 72°C for 45 seconds followed by a final elongation step at 72°C for 7 minutes and hold temperature at 10°C. Amplified fragments were visualized on Safe view-stained 1.5% agarose electrophoresis gels using Hyper ladder 1 DNA marker.

Base Sequence Analysis

The amplicons were subjected to sequencing reactions using BigDye Terminator v3.1 Cycle Sequencing Kit. The products were loaded unto 3130xl Genetic Analyzer (Applied Biosystems).

The base sequences generated from amplicon were edited using Bioedit v6 application. Sequences were submitted as query at for comparison with database sequences using the NCBI nucleotide BLAST. Isolates were identified based on DNA-DNA homology at >90%.

Statistical Analysis

Chi square test was used for assessing the statistical significance of data generated at $P < 0.05$.

Results and discussion

Table 1 shows the distribution of salad types in 85 samples from fast food, single vegetables was 15 which was 17.6% being the lowest of the salad types. The highest number of samples of 50 (58.8%) was salad with dressing. In distribution of the 85 samples from the street vendors, samples from mixed vegetables was 5 (5.9%), followed by single vegetables with 25 (29.4%) samples and the highest number of samples recorded was 55 (64.7%) for salad with dressing. The lowest type of sample from both the fast food and street vendors was mixed vegetables recorded at 25 (14.7%) followed by single vegetables 40 (23.5%) and Salad with dressing 105 (61.8%) all of which amounted to a total of 170 (100%). A significant difference (P=0.021) was observed between the source of samples and the salad types.

Table 1. Distribution of salad types in samples collected from fast food and street vendors.

Salad Types	Source of samples		Total	χ^2	P<0.05
	Fast Food (%)	Street Vendors (%)	(%)		
Single Vegetables	15 (17.6)	25 (29.4)	40 (23.5)	11.7	0.021
Mixed Vegetables	20 (23.5)	5 (5.9)	25 (14.7)		
Salad with dressing	50 (58.8)	55 (64.7)	105 (61.8)		
Total	85 (100)	85 (100)	170 (100)		

Table 2 shows the percentage of compliance based on total viable count and total coliform count of the different types of salad samples based on FSANZ (Food Standards Australia New Zealand, 2010). The presence of the pathogen *Salmonella* spp. in the samples was also evaluated in reference to the guidelines. In reference to total viable count: 38 (95%) were satisfactory while 2 (5%) were unsatisfactory for the 40 single vegetables that were investigated; 28 (93.3%) were satisfactory while 2 (6.7%) were unsatisfactory of 30 mixed vegetables; while 96 (96%) were satisfactory and 4 (4%) was unsatisfactory of the 100 salad with dressing samples investigated. There was a significant difference (P= 0.024) in the levels of total viable count among the different types of samples, having salads with dressing recording the highest. In reference to total coliform count: 37 (92.5%) were satisfactory while 3 (7.5%) were not from the single vegetables; 27 (90%) were satisfactory while 3 (10%) were not of the mixed vegetables; while 97 (97%) were satisfactory and 3 (3%) were unsatisfactory of the salad with dressing. There was a significant difference among samples with regards to total coliform count (P=0.03), having salads with dressing recording the highest. From the evaluation of *Salmonella* spp. in the samples, 24 (60%) single vegetables samples was satisfactory because *Salmonella* was not detected in them but 16 (40%) were not; 12 (40%) mixed vegetables samples were satisfactory, while 18 (60%) were not; and 68 (68%) were free of *Salmonella* while 32 (32%) were not of the salad with dressing samples. There was a significant difference with respect to the presence of *Salmonella* in the different samples (P=0.014), with salads with dressing recording the highest.

Table 3 shows the microbial compliance of the samples in relation to sample sources. In terms of total viable count: 82 (96.5%) were satisfactory from fast food outlets while 3 (3.5%) were not; and 80 (94.1%) were satisfactory while 5 (5.9%) were unsatisfactory of the samples from

street vendors. There was a significant difference among sample sources and total viable count (P=0.032). In regards to total coliform count, 82 (96.5%) samples from fast food were satisfactory while 3 (3.5%) were unsatisfactory and 79 (93%) were satisfactory while 6 (7%) were unsatisfactory from the street vendors.

There was a significant difference among sample sources and total coliform count (P=0.025), with samples from street vendors recording higher. In reference to the detection of *Salmonella* in all samples, 58 (68.2%) of the samples from the fast food were satisfactory while 27 (31.8%) were unsatisfactory; and 52 (61.2%) samples from the street vendors were satisfactory while 33 (38.8%) were unsatisfactory. There was a significant difference among sample sources in reference to presence of *Salmonella* (P=0.016), with samples from street vendors recording high.

Table 2. The percentage of compliance of salad samples based on various microbiological parameters.

Parameters	Compliance with guidelines for microbiological quality	Salad Type				χ^2	P<0.05
		Single	Mixed	Salad with dressing	Total		
TVC	S	38 (95)	28 (93.3)	96 (96)	162 (95.2)	15.2	0.024
	U	2 (5)	2 (6.7)	4 (4)	8 (4.7)		
Total		40 (23.5)	30 (17.6)	100 (58.8)	170 (100)		
TCC	S	37 (92.5)	27 (90)	97 (97)	161 (94.7)	13.3	0.03
	U	3 (7.5)	3 (10)	3 (3)	9 (5.2)		
Total		40 (23.5)	30 (17.6)	100 (58.8)	170 (100)		
Indicator Organism							
Salmonella	S	24 (60)	12 (40)	68 (68)	104 (61.2)	14.7	0.014
	U	16 (40)	18 (60)	32 (32)	66 (38.8)		
Total		40 (23.5)	30 (17.6)	100 (58.8)	170 (100)		

Key: S= Satisfactory U= Unsatisfactory TVC= Total Viable Count TCC= Total Coliform Count.

Table 3. The compliance percentage of salad samples according to sample sources.

Parameters	Compliance with guidelines for microbiological quality	Source of Samples		χ^2	P<0.05
		Fast Food	Street Vendors		
TVC	S	82 (96.5)	80 (94.1)	9.6	0.032
	U	3 (3.5)	5 (5.9)		
Total		85 (100)	85 (100)		
TCC	S	82 (96.5)	79 (93)	8.4	0.025
	U	3 (3.5)	6 (7)		
Total		85 (100)	85 (100)		
Indicator Organism					
Salmonella	S	58 (68.2)	52 (61.2)	12.3	0.016
	U	27 (31.8)	33 (38.8)		
Total		85 (100)	85 (100)		

Key: S= Satisfactory U= Unsatisfactory TVC= Total Viable Count TCC= Total Coliform Count.

Table 4 showed that all isolates, from the three different sources (fast food, street vendors and clinical isolates), were resistant at 100% to Ceftazidine 30µg, Cefuroxime 30µg and Augmentin 30µg. Fast food isolates also showed resistance to Cefixime 5µg at 97%. The clinical isolates however were resistant to Ofloxacin 5µg and Ciprofloxacin 5µg at 55% and 40% respectively, while those from fast food and street vendors had 0 to 3% resistance. Clinical isolates and street vendors had 25% and 2.1% resistance respectively to Gentamicin 10µg. Nitrofurantoin 300µg showed the best potency with the lowest resistance percentages of clinical isolates at 20%, street vendor isolates at 2%, and fast food isolates at 0%. In table 5, the resistance pattern of Cefixime-Ceftazidine-Cefuroxime-Augmentin occurred in the clinical

isolates and vegetable salads from fast foods and street vendors. The vegetable salads from street vendors had the highest number of isolates with this resistance profile at 44 (51.8%), followed by those from fast food 30 (35.3%). However, a very percentage of resistance was observed in the clinical isolates at 19 (95%). The resistance pattern of Ceftazidine-Cefuroxime-Augmentin was also found in the clinical isolates and isolates from vegetable salads from the different sources.

Table 4. Percentage antibiotics resistance of isolates from the different sources of vegetable salads and clinical isolates.

	Resistance of Isolates (%)		
Antibiotics (µg)	Fast food	Street vendors	Clinical isolates
Ceftazidine (30)	100	100	100
Cefuroxime (30)	100	100	100
Gentamicin (10)	0	2.1	25
Cefixime (5)	97	0	0
Ofloxacin (5)	3	0	55
Augmentin (30)	100	100	100
Nitrofurantoin (300)	0	2	20
Ciprofloxacin (5)	0	0	40

The clinical isolates had the highest number of isolates with the resistance profile while isolates from vegetables salads from the different sources both had 1 (1.2%). Ofloxacin-Cefixime-Ceftazidine,-Cefuroxime-Augmentin resistance pattern was found in only 1 (1.2%) isolate from fast food vegetable salads; also 1 (1.2%) isolate from vegetable salads from street vendors had the resistance pattern Cefixime-Nitrofurantoin-Ceftazidine-Cefuroxime-Augmentin.

Table 5. Antimicrobial resistance profile of isolates from the different sources.

Antimicrobial resistance pattern	No. of resistant isolates with the same resistant pattern.		
	Fast food (%)	Street vendors (%)	Clinical isolates (%)
Cxm-Caz-Crx-Aug	30 (35.3)	44 (51.8)	19 (95)
Caz-Crx-Aug	1 (1.2)	1 (1.2)	1 (5)
Gen-Cxm-Caz-Crx-Aug	1 (1.2)	1 (1.2)	0
Ofl-Cxm-Caz-Crx-Aug	1 (1.2)	0	0
Cxm-Nit-Caz-Crx-Aug	0	1 (1.2)	0

Key: (Caz)-Ceftazidine 30µg, (Crx)-Cefuroxime 30µg, (Gen)-Gentamicin 10µg, (Cxm)-Cefixime 5µg, (Ofl)-Ofloxacin 5µg, (Aug)-Augmentin 30µg, (Nit)-Nitrofurantoin 300µg, (Cpr)-Ciprofloxacin 5µg.

Plate 1 showed a photograph of DNA bands of 13 representative isolates after the quantification by gel electrophoresis. The clinical isolates were coded C13, C14, C17, C18, isolates from vegetable salads fast food origin were coded S2, S7, S45, S127 and isolates from vegetable salads street vendors were coded S8, S12, S76, S120 and S90. Plate 2 shows the photograph of 16S rRNA amplified bands of 10 representative isolates and a ladder taken under ultraviolet light after gel electrophoresis.

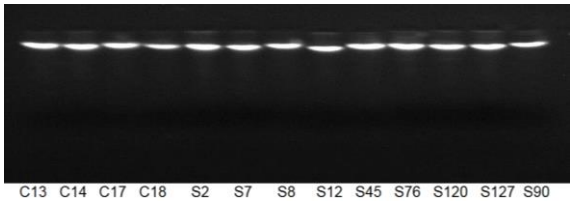

Plate 1. Deoxyribonucleic acid of 13 representative isolates.

Key: C13, C14, C17, C18 (Clinical isolates); S2, S7, S45, S127 (fast food); S8, S12, S76, S120, S90 (street vendors).

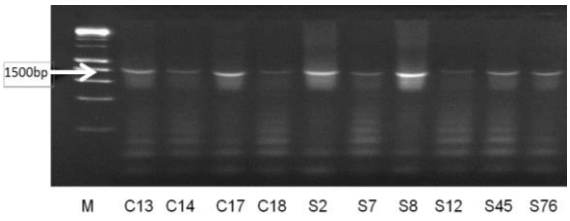

Plate 2. Ethidium-bromide stained agarose gel of amplified 16S rRNA bands some isolates.

Key: M= Ladder; C13, C14, C17, C18 (Clinical isolates); S2, S7, S45, S127 (fast food);] S8, S12, S76, S120, S90 (street vendors).

Table 6 showed the BLAST results of NCBI database homologues with isolates from this study. Isolates Sam 45 and Sam 127 from vegetable salads from fast food and isolates Sam 120 and Sam 90 from vegetable salads from street vendors were all homologues of *Salmonella enterica* subsp. enterica strain LT2; isolates Sam 2 and Sam 7 from vegetable salads from fast food and isolate Sam 12 from vegetable salad from street vendor were homologues of *Salmonella enterica* subsp. enterica serotype

Typhimurium strain ATCC 1311; clinical isolates C13 and C18 were homologues of *Salmonella enterica* subsp. indica strain DSM 14848; isolates C14 and C17 were homologues of *Salmonella enterica* subsp. houtenae strain DSM 9221; Sam 8 from vegetable salads obtained from street vendor recorded high homology with *Salmonella enterica* subsp. enterica strain ABUH7; and Sam 76 from street vendor samples was homologous to *Salmonella enterica* strain 55.12.1.

Table 6. Blast hits of representative isolates.

Isolates	Identity (%)	*Salmonella* Species	Accession No
Clinical isolates			
C13	93.99	*Salmonella enterica* subsp. indica strain DSM 14848	NR044370.1
C14	91.41	*Salmonella enterica* subsp. houtenae strain DSM 9221	NR 044371.1
C17	94.06	*Salmonella enterica* subsp. houtenae strain DSM 9221	NR 044371.1
C18	96.36	*Salmonella enterica* subsp. indica strain DSM 14848	NR044370.1
Isolates from vegetable salads obtained from fast food outlets			
Sam 2	93.60	*Salmonella enterica* subsp.enterica serotype typhimurium strain ATCC 1311	NR116126.1
Sam 7	90.09	*Salmonella enterica* subsp.enterica serotype typhimurium strain ATCC 1311	NR116126.1
Sam 45	94.49	*Salmonella enterica* subsp.enterica strain LT2	NR074910.1
Sam 127	92.81	*Salmonella enterica* subsp.enterica strain LT2	NR074910.1
Isolates from vegetable salads obtained from street vendors			
Sam 8	91.84	*Salmonella enterica* strain ABUH7	MH817559.1
Sam 12	90.89	*Salmonella enterica* subsp.enterica serotype typhimurium strain ATCC 1311	NR119108.1
Sam 76	90.42	*Salmonella enterica* strain 55.12.1	MG859622.1
Sam 120	91.98	*Salmonella enterica* subsp.enterica strain LT2	NR074910.1
Sam 90	93.65	*Salmonella enterica* subsp.enterica strain LT2	NR074910.1

Salmonella is of public health concern due to the fact that most strains are potentially pathogenic, causing morbidity and mortality (Lutful, 2010) in both humans and animals worldwide. It has been reported to cause life-threatening systemic infections, especially among high risk persons such as infants, children, elderly and immuno compromised individuals (Idowu *et al*., 2017). In this study, a percentage of 4.7 (8/170) of the RTE salads from both the fast food and street vendors below standard preparation guidelines for microbiological quality, that is, they were ≥ 10^5 CFU/g total viable count. A similar trend has been reported with a minority of samples failing the microbial load test (Erkan and Vural, 2008). The level of total viable count in all vegetables salad samples ranged from 10^3 to 10^6 CFU/g. Similar findings had been reported in other studies; in Turkey at 10^2 to 10^6 CFU/g (Aycicek *et al*., 2006; Elmacioglu *et al*., 2010), and in Egypt, at $<10^6$ CFU/g (Halablab *et al*., 2011). A different report from Sudan and Nigeria however, ranged from 10^5 to 10^8 CFU/g (Abdullahi and Abdulkareem, 2010; Goja and Mahmoud, 2013), a little higher than the results from this study. While in Spain, a study reported that total viable count ranged from 2.7×10^5 to 8.0×10^6 CFU/g in whole fresh vegetables and from 4.3×10^5 to 8.9×10^7 CFU/g in fresh-cut vegetables (Abadias *et al*., 2008). It was observed that there was a statistically significant difference of (P= 0.024) in the levels of total viable count among the different types of samples. These differences were probably due to the cultivation areas of the vegetables, type of manures used, transportation condition, poor storage conditions and holding temperatures at point of sales as well as personal hygiene (such as washing of vegetables, hand contact) (Abe *et al*., 2016).

The percentage of salad vegetables samples that failed to comply with standards of total coliform count was 5.2% (9/170) at ≥ 10^4 CFU/g, while 94.7% (161/170) of the samples were found to be compliant with a count $<10^4$ CFU/g. All the different types of vegetables salads showed the

same number of non-compliant isolates but with different percentages. Single vegetables 3 (7.5%), mixed vegetables 3(10%) and salad with dressing 3(3%). In this study it was found that total coliform count ranged from 1×10^2 to 2×10^6 CFU/g which agrees with a study reported in Turkey at 1.2×10^3-1.8×10^5 CFU/g (Elmacioglu *et al.,* 2010). In the study carried out in Lebanon (Halablab *et al*., 2011), it was found that the level of total coliform count in all fresh vegetable samples ranged from 2.0 to 10.71 log10 CFU/g. In another study from Nigeria on ready to eat leafy vegetables the total coliform count ranged from 8.0 x 10^5 to $1.3\text{x}10^9$ CFU/g (Abdullahi and Abdulkareem, 2010).

Salmonella spp. was present in 38.8% (66/170) of the vegetable salad samples. Percentage of *Salmonella* spp. was found as follows; 60% (18/30) of the mixed vegetables salad samples, 40% (16/40) of single vegetables salad and 32% (32/100) of vegetables salad with dressing. In other studies, different findings has been reported, a study in India reported (5.8%) of *Salmonella* spp. in different vegetables salad (Avazpour *et al*., 2013)**.** In Nigeria, a study reported (6.7%) *Salmonella* spp. in vegetables salad **(**Itohan *et al*., 2011) and in studies carried out in Iran it was reported that *Salmonella* spp. was 5.6% in mixed fresh salads and in another 9.4% of *Salmonella* in mixed green leaves vegetables (Mohammad *et al*., 2012). The difference in the results could be attributed to the differences in the amount of samples investigated, which was higher than of this study. Also another reason why the presence of *Salmonella* spp. was higher in this study could be from washing vegetables with contaminated water, handling of vegetables by carrier, infected workers, contaminated irrigation water with sewage and organic manures widely used by farmers in fertilization of vegetables (Berger *et al*., 2010). Vegetable salad samples from both the fast food and street vendors were contaminated with *Salmonella enterica* subsp.enterica serotype typhimurium.

This was similar to the bacterial contamination of ready-to-eat vegetable salad in study carried out in Iran by Kochakkhani *et al*. (2018), contamination of raw vegetable salads obtained in Abidjan, Côte d'Ivoire (Toe *et al*., 2017). These studies were in agreement with this study regarding the presence of *Salmonella enterica* subsp. enterica serotype typhimurium in ready-to-eat vegetable. Among the clinical isolates, *Salmonella enteritidis* was predominant, this was similar to the study by Kochakkhani *et al*. (2018) in which *Salmonella enteritidis* was dominant among other *Salmonella* species. The strains of *Salmonella* obtained from the clinical isolates after molecular analysis were *Salmonella enterica* subsp. indica and *Salmonella enterica* subsp. houtenae. This differed from the results obtained from the study by John *et al*. (2017) and Aasia *et al*. (2009) whereby the isolates were characterized and identified as *Salmonella enterica* serovar Typhimurium after molecular analysis from clinical samples. In a study by Biserka *et al*., (2019) after molecular analysis, all the *Salmonella* spp. from vegetable salads belonged to serovar Enteritidis, this was similar to another study by Fiedler *et al.,* (2017) in which all isolates belonged to serovar Enteritidis. These reports differ from this present study as no isolates from vegetable salads belonged to the serovar Enteritidis. Therefore results from studies carried out by Biserka *et al*., (2019) and Fiedler *et al.,* (2017) are different from this study.

Although all *Salmonella* strains isolated in this study were tested for their susceptibility to eight antibiotics, resistance to Ceftazidine (100%), Cefuroxime (100%) and Augmentin (100%) were detected predominantly from all sources. Most of the isolates from fast food were resistant to Cefixime (97%) while clinical isolates and isolates from vegetable salads from street vendors were not. Nitrofurantoin had the lowest resistance recorded among all the isolates, only 20% of isolates clinical isolates and 2% of the isolates from vegetable salads from street vendors were resistant to it.

The results from the study were similar to that in the study by Birce *et al*., (2013) where the isolates were resistant to Ceftazidine (90%), Cefuroxime (90%) and Augmentin (90%). The study also had Nitrofurantoin (5%) recording the lowest resistance among the isolates. Ciprofloxacin had the highest level of susceptibility in the study by (Gordana *et al*., 2010) it also susceptible in this study therefore it shows that both studies agree that Ciprofloxacin is good for treatment of infection by *Salmonella.* Five antibiotic resistance profile was identified in this study. The antibiotic resistance profile Cefixime-Ceftazidine-Cefuroxime-Augmentin was found in the isolates from the different sources (vegetable salads from fast food, vegetable salads from street vendors and clinical isolates). Three of the resistance pattern found in this study was also found in the study by Guerra *et al*., (2004) indicating that it agrees with this study. Therefore, this shows the rapid emergence of antibiotic resistant pathogens, which is becoming a growing public health concern all over the world.

The antimicrobial resistance observed in the present study is probably due to the widespread use of the commonly available antimicrobials.

Molecular characterization of *Salmonella* species identified *Salmonella enterica* subsp. indica strain DSM 14848, *Salmonella enterica* subsp.enterica strain LT2, *Salmonella enterica* subsp.enterica serotype *typhimurium* strain ATCC 1311, *Salmonella enterica* subsp. houtenae strain DSM 9221 and *Salmonella enterica* subsp.enterica strain Ty2. The sequences were homologous to each other but the homology was low, only one sequence had above 95% similarity. The representative sequences in this study were not 100% similar to the sequences in the NCBI database which could suggest new and different sequences from those in the database. This is similar to the study by Hyun-Joong *et al*., (2006) in which 12 genomic sequences were compared with *Salmonella* subspecies and only one had 96% similarity with *Salmonella* serovar Typhimurium LT2. This therefore brings to the fore the importance of using specific primers in the amplification of bacterial isolates' DNA. The conserved and variable regions of the 16S rRNA gene from the clinical isolates were compared with the isolates from the vegetable salads. Isolates from the fast food vegetable salads showed a closer relationship to the clinical isolates by having 48% similarity between their conserved regions and 47% similarity in their variable regions while isolates from the vegetable salads street vendors had 28% conserved regions and 67% variable regions. These goes on to show the relationship and similarities between the clinical isolates and isolates from ready-to-eat vegetable salads. This result is similar to results from the study by Rebecca *et al*., (2016) in which 16S rRNA gene *Salmonella* from ready-to-eat produce was compared with 16S rRNA gene from clinical isolate and revealed the percentage of the relationship between their conserved regions. The phylogenetic tree (not shown) in this study had two clusters one cluster contained all the representative isolates while the other contained the sequences from NCBI database, this showed that the representative isolates had a common ancestor. The sequences in the second clade were closely clustered showing that they were very closely related, this is similar to the study by Hyun-Joong *et al*., (2006) where the phylogenetic tree also had two clades and the sequences which were from NCBI database in the second clade were very closely related.

Conclusion

Ready-to-eat salads from fast food and street vendors were considered not safe because of the presence of *Salmonella*. The clinical isolates and isolates from vegetable salads from fast food and street vendors comprised of *Salmonella enterica* indicating the widespread of the isolate across all samples. Overall it was evident that humans played a major role in the contamination of vegetable salads during distribution and preparation.

The antibiotics classes of cephalosporins and β-lactamase inhibitors were not suitable for treating *Salmonella* infections due to the resistance of *Salmonella* species to these classes of antibiotics.

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References

Aasia B, Asma H, Samina B, Aamir A, Yasra S, Abdul H. 2009. Molecular analysis of drug resistance in clinical isolates of MDR *Salmonella enterica* Serovar Typhi in Faisalabad, Pakistan. Pakistan Journal of Zoology **41,** 363-369.

Abadias M, Usall J, Anguera M, Solsona C, Vinas I. 2008. Microbiological quality of fresh, minimally processed fruit and vegetables and sprouts from retail establishments. International Journal of Food Microbiology **123,** 121-129.

Abdullahi O, Abdulkareem S. 2010. Bacteriological quality of some ready-to-eat vegetables as retailed and consumed in Sabon-Gari, Zaria, Nigeria. Bayero Journal of Pure and Applied Sciences **3,** 173-175.

Abe K, Jelalu K, Haile A, Solomon HM. 2016. Isolation, identification and antibiotic susceptibility testing of *Salmonella* from slaughtered bovines and ovines in Addis Ababa Abattoir Enterprise, Ethiopia: A cross-sectional Study. International Journal for Microbiology **16,** 205-212.

Aboh MI, Oladosu P, Ibrahim K. 2011. Bacterial contaminants of salad vegetables in Abuja Municipal Area Council, Nigeria. Malaysian Journal of Microbiology **7,** 111-114.

Angela OE, Ibukunoluwa A O, Oranusi US. 2010. Microbial quality of fruits and vegetables sold in Sango Ota, Nigeria. African Journal of Food Science **4,** 1-5.

Applied Biosystems. (2010). BigDye Terminator v3.1 Cycle Sequencing kit protocol. Available [Online] at www.appiedbiosystems.com.

Arowosegbe S, Olanipekun KM, Adeloye AI 2018. Ethnobotanical survey of indigenous leafy vegetables consumed in Ekiti State, Nigeria. European Journal of Biology and Medical Science Research **6,** 7-14.

Avazpour M, Nejad M, Seifipour F, Abdi J. 2013. Assessment of the microbiological safety of salad vegetables from different restaurants in Ilam. Journal of Paramedical Sciences **4,** 111-115.

Aycicek H, Oguz U, Karci K. 2006. Determination of total aerobic and indicator bacteria on some raw eaten vegetables from wholesalers in Ankara, Turkey. International Journal of Hygiene and Environmental Health **209,** 197-201.

Berger C, Sodha S, Shaw R, Griffin P, Pink D, Hand P, Frankel G. 2010. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. Environmental Microbiology **12,** 2385-2397.

Biserka B, Dominic S, Patrick S, Sabine K, Melanie H. 2019. Microbial contamination of organically and conventionally produced fresh vegetable salads and herbs from retail markets in southwest Germany. Foodborne Pathogens and Disease **16,** 4-10.

Bull MJ, Marchesi JR, Vandamme P, Plummer S, Mahenthiralingam E. 2012. Minimum taxonomic criteria for bacterial genome sequence depositions and announcements. Journal of Microbiological Methods **89,**18-21.

Chun J, Rainey FA. 2014. Integrating genomics into the taxonomy and systematics of the bacteria and archaea. International Journal of Systematic and Evolutionary Microbiology **64,** 316-324.

City population worldwide. (2018). Available Online from http:population.city/nigeria/adm/ekiti/ Accessed on February 22, 2019.

Coorevits L, Boelens J, Claeys G. 2015. Direct susceptibility testing by disk diffusion on clinical samples: a rapid and accurate tool for antibiotic stewardship. European Journal of Clinical Microbiology and Infectious Diseases **34,** 1207-1212.

David OM, Adedapo DA, Moro DD, Esan CO, Oje OJ, Famurewa O. 2015. Prevalence and carriage status of *Salmonella typhi* among students of Ekiti State University, Ado-Ekiti, Nigeria. International Journal of Biosciences **6,** 1-8.

Erkan M, Vural A. 2008. Investigation of microbial quality of some leafy green vegetables. Journal of Food Technology **6,** 285-288.

Farahnaaz F, Jessica DS, Rashed N. 2013. Determination of microbial growth and survival in salad vegetables through *in-vitro* challenge test. International Journal of Nutrition and Food Sciences **2,** 312-319.

Farjana R, Rashed N. 2012. Prevalence of pathogenic bacteria in common salad vegetables of Dhaka Metropolis. Bangladesh Journal of Botany **41,** 159-162.

Food Standards Australia New Zealand (2010). Guidelines for the microbiological examination of ready to eat foods. Retrieved March 2019 from http://www.foodstandards.gov.au/_srcfiles/Guidelines %20for%20Micro%20exam.pdf.

Garcia-Sepulveda CA, Carrillo-Acuna E, Guerra-Palomares SE, Barriga-Moreno M. 2010. Maxiprep genomic DNA extractions for molecular epidemiology studies and biorepositories. Molecular Biology Reports **37,** 1883-1890.

Goja A, Mahmoud M. 2013. Microbial quality of some vegetables sold in ED Dueim Twon, Sudan. Pakistan Journal of Biological Sciences **16,** 585-588.

Gordana M, Bogdanka A, Dragica T, Milena L, Brankica D. 2012. Antibiotic susceptibility of *Salmonella* spp.: A comparison of two surveys with a 5 years interval. International Journal for Microbiology **18,** 216-210.

Guerra B, Junker E, Miko A, Helmuth R, Mendozamc. 2004. Characterization and localization of drug resistance determinants in multi-resistant, integrin carrying *Salmonella enterica* serotype Typhimurium strains. Microbial Drug Resistance **10,** 83-91.

Halablab M, Sheet I, Holail H. 2011. Microbiological quality of raw vegetables grown in Bekaa Valley, Lebanon. American Journal of Food Technology **6,** 129-139.

Hyun-Joong K, Si-Hong P, Hae-Yeong K. 2006. Comparison of *Salmonella enterica* Serovar Typhimurium LT2 and Non-LT2 S*almonella* genomic sequences, and genotyping of *Salmonellae* by using PCR. Applied and Environmental Microbiology **72,** 6142-6151.

Idowu OF, Lisa B, Marzia M, Jacob K, Sati SN, Paola Z, Antonia, AL, Monica L, Paul AA, Junaidu K, Jarlath U, Antonia R, Maryam M. 2017. *Salmonella* serovars and their distribution in Nigerian commercial chicken layer farms. Public Library of Science **12,** 3-9.

Itohan A, Peters O, Kolo I. 2011. Bacterial contaminants of salad vegetables in Abuja Municipal Area Council, Nigeria. Malaysian Journal of Microbiology **7,** 111-114 (2011).

Joana S, Daniela L, Mariana F, Cristina M, Paul AG, Paula T. 2011. *Campylobacter* spp. as a foodborne pathogen: A Review. Frontiers in Microbiology **2,** 200-208.

John M, Roberta B, Caroline H, Judith C, Tim D, Philip A, Robert J, Deborah M. 2017. Investigation using whole genome sequencing of a prolonged restaurant outbreak of *Salmonella typhimurium* linked to the building drainage system, England, February 2015 to March 2016, Eurosurveillance **22,** 17-37.

Kochakkhani H, Dehghan P, Moosavy MH. 2018. Molecular detection of *Salmonella enterica* Serovar Typhimurium in ready-to-eat vegetable salads consumed in restaurants of Tabriz, North-West of Iran. Journal of Food Quality and Hazards Control **5,** 140-145.

Lutful KSM. 2010. Avian Colibacillosis and Salmonellosis: A closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. International Journal of Environmental Research and Public Health **7,** 89-100

Malik AH. 2016. Food contamination: major challenges of the future. Molecular Diversity Preservation International and Multidisciplinary Digital Publishing Institute **5,** 1-2.

Mircea VM, Lorena C, Olga H O, Ioana P, Teodora A, Vasile N. 2016. Semiology of food poisoning. Human and Veterinary Medicine International Journal of the Bioflux Society **8,** 108-111.

Mohammad B, Najafi H, Bahreini M. 2012. Microbiological quality of mixed fresh-cut vegetable salads and mixed ready-to-eat fresh herbs in Mashhad, Iran. International Proceedings of Chemical, Biological and Environmental Engineering Journal **39,** 62-66.

Rebecca LB, Karen GJ, Andrea RO, Melinda AM, Eric WB. 2016. Recent and emerging innovations in *Salmonella* detection: a food and environmental perspective. Microbial Biotechnology **9,** 279-292.

Reza M, Hamid B, Mehrdadmm, Samaneh K, Fatemeh S. 2012. Rapid DNA extraction of bacterial genome using laundry detergents and assessment of the efficiency of DNA in downstream process using polymerase chain reaction. African Journal of Biotechnology **11,** 173-178.

Scallan E, Griffin PM, Angulo FJ, Tauxe R V, Hoekstra RM. 2011. Foodborne illness acquired in the United States unspecified agents. Emerging Infectious Diseases **17,** 16-22.

Sowjanya M. 2016. Food Poisoning: Mini-review. Research and Reviews Journal of Pharmaceutical Analysis **5,** 136-141.

Toe E, Dadié A, Dako E, Loukou G. 2017. Bacteriological quality and risk factors for contamination of raw mixed vegetable salads served in collective catering in Abidjan (Ivory Coast). Advances in Microbiology **7,** 405-41.