Molecular Detection of Virulence Genes in *Salmonella* Spp Isolated From Ready to Eat Fruits Salad Sold In Plateau and Kogi North Central, Nigeria

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ABSTRACT

Salmonella species are known to be public health trial in Nigeria. *Salmonella* species contaminations in ready-to-eat fruit salads are becoming progressively popular. This study aimed at molecular detection of virulence genes in *salmonella* sp isolated from ready to eat fruits salad in some selected state in North Central, Nigeria. A total of 300 ready-to-eat fruit-salads samples were randomly collected from major Markets in Plateau and Kogi. Isolation and identification of salmonella sp were carried out using standard microbiology methods. Antibiotic susceptibility testing was performed by disk diffusion using Clinical and Laboratory Standards Institute method. Virulence genes detection was carried out using polymerase chain reaction boiling technique. Out of 300 ready-to-eat fruit-salads collected, the overall occurrence of *salmonella* spp 12 (4.0%) and the highest occurrence was recorded from Plateau state (8.0%), and Kogi state (4.0%). The antibiotics resistance of *Salmonella* sp isolated from ready to eat fruit salads sold in Plateau state showed that *Salmonella* sp were highly resistance to cefexime (50.0 %)but less resistance to gentamicin and amoxicillin/ clavulanic acid (12.5 %). From Kogi the order of percentage resistance of *Salmonella species* to the antibiotics tested were, nalidixic acid (75.0 %) and amoxicillin/ clavulanic acid (25.0 %). The commonest MAR in *Salmonella* sp isolated from Plateau was 0.4 with percentage of occurrence of 37.5 %. The commonest MAR in *Salmonella* species isolated from Kogi was 0.3 with percentage of occurrence of 50.0 %. The order of virulence genes detected were, *spiA* genes (33.3 %). From *Salmonella* species isolated from Plateau harbors *spiA* (66.6 %). From *Salmonella* species isolated from Kogi harbors *spiA* (33.3 %). In this study it was observed that the ready to eat fruit-salads sold in major cities in North Central Nigeria were contaminated with *Salmonella* and is resistance to commonly use antibiotic in treatment of infection cause by these *Salmonella.*

Keywords: Foodborne illnesses, antibiotics resistance, *Salmonella* sp, ready-to-eat fruit salads

INTRODUCTION

Fruits have been greatly recommended in foods due to their health- promoting attributes. They are very essential in dietary guidance since they contain high levels of minerals particularly electrolytes, vitamins such as vitamin C, and phytochemicals which act as anti- oxidants and fibres [1. Therefore, the consumption of fresh fruits or their juices is crucial to healthy living [2]. In addition, it is expected that a rich balanced diet should consist of fruits due to their numerous functions. Some of which include the ability to develop a blood lipid profile, detoxify the human body, and prevent vitamin deficiencies [3, 1). Also, the consumption of sufficient amounts of fruits lowers blood cholesterol levels, controls blood pressure, reduces the risk of some heart diseases, and prevents some kinds of cancer [4]. When fruits are harvested or during post-harvest processing, they come in contact with water, soil, dust, and handling. Consequentially, they harbour a lot of microorganisms including pathogens [5]. Also, harvested fruits are commonly sold as cut or sliced fruits to entice the consumers.

These types of fruit are referred to as vended fruits or ready-to-eat fruits, some of which include watermelons, pineapples, cucumbers, mangoes, oranges, pawpaws, etc. They are usually displayed at strategic places or carried around by hawkers to be sold to buyers for immediate consumption without necessarily rinsing or washing them because they have already been prepared and packaged in small polythene bags [6].

The consumption of these vended ready-to-eat fruits has become a global trend. This is due to their accessibility, convenience, and relatively cheaper prices than the whole fruits [7]. Thus, they have gradually become staples due to the recent modernization, industrialization, economic downturn, materialism, and unavailability of ample time to prepare a proper meal in some homes [8]. The increase in consumption of vended fruits has been reported to signal a great risk to consumer health because it is difficult to ascertain the hygienic processes the fruits are subjected to after harvesting, during processing, and before packaging [8]. In addition, pathogens could invade the fruits during washing, peeling, dicing, trimming, packaging, handling, and marketing [9]. This is aggravated by the fact that the preparation of vended fruits is done without proper storage conditions, thereby exposing them to heat, flies, cockroaches, rodents, dust, pathogens, dirt, and other environmental contaminants [9]. This study aimed at molecular detection of virulence genes in *Salmonella* spp isolated from ready to eat fruits salad sold in Plateau and Kogi North Central, Nigeria

MATERIALS AND METHODS

Study Area

The study was conducted in two selected States in North Central, Nigeria, one of the six geopolitical zones in Nigeria. This study areas comprised Plateau and Kogi,. Based on projections from the 2006 National Population Census, North Central Nigeria is home to an estimated 29.8 million people National Population Commission, 2006. The area is characterized by a blend of urban centers, peri-urban settlements, and rural communities, with vibrant markets and street vending systems that supply fresh fruits and ready-to-eat (RTE) products to a large population. [10]

Sample Collection

A total of two hundred (200) fruit salads samples were ramdomly collected from hawkers on the streets and markets across the tow selected states in North Central Nigeria (Plateau and Kogi), with hundred (100) samples from each state. The samples were transferred into sterile sample bottles. Samples were labeled according to sampling points (markets and streets) and transported to the Department of Microbiology laboratory in Nasarawa State University, Keffi in a sterile and sealed plastic ice bag for immediate analysis.

Preparation of Media and Inoculation for the Isolation of Salmonella Species

Preparation of Culture media:

2.3.1 Nutrient Broth: thirteen grams of dehydrated power was dispensed into one liter of distilled water, boiled to completely dissolved and sterilized by autoclaving at 15 lbs pressure (121 ^{o}C) for fifteen minutes [11].

2.3.2 Salmonella Shigella Agar: Sixty grams of medium was weighed and dispensed in one liter of deionized water, mixed and heated with frequent agitation and boiled for one minute without autoclaving. It was poured into sterile petri dishes and allowed to solidified [11].

2.3.3 Xylose lysine Deoxycholate: Fifty-five grams of dehydrated medium was suspended in one liter of distilled water and heated with agitation until it boiled, then transferred to a 50 ^{o}C water bath. It was dispensed into sterile petri dish [11].

2.3.4 Bismuth Sulphite Agar: A fifty- two point thirty-three grams of the dehydrated medium was suspended into distilled water and was heated to boil to dissolve completely. The suspension was dispensed into petri dishes to solidify [11].

Isolation of *Salmonella* species

Salmonella species were isolated using a modified protocol from Bako *et al.* [12]. Approximately 25 g of each fruit salad sample was homogenized in a sterile blender and transferred to 25 mL of Nutrient Broth (Oxoid, UK) for pre-enrichment. Samples were incubated aerobically at 37 °C for 24 hours. Post-enrichment, 1 mL was serially diluted (10^{-3} and 10^{-5}), and 0.1 mL was spread-plated in duplicate onto Xylose Lysine Deoxycholate (XLD) agar, *Salmonella*-Shigella Agar (SSA). Plates were incubated at 37 ^{O}C for 24 hours. Presumptive *Salmonella* colonies—typically appearing as red colonies with black centers on XLD or colorless colonies with black centers on *Salmonella Shigella* Agar The suspected *Salmonella* species on Salmonella shigella Agar plates were streaked onto Bismute sulphite agar plates and incubated at 37 ^{o}C for 24 hours for presumptive confirmation of Salmonella species. Salmonella species colonies with a dark metallic sheen caused by large quantities of acid that is produced onto the growth surface

Identification of *Salmonella* species

The *Salmonella* species isolates were further identified using standard microbiological procedures based on cultural, morphological and biochemical characteristics namely Indole Test, Methyl Red Test/Voges-Proskauer Test and Citrate Test

Analytical Profile Index 20E (API 20E) kit

The API 20E kit was used for definitive identification of *Salmonella* serovars as Enterobacteriaceae. A bacterial suspension from a pure *Salmonella* colony was prepared in sterile saline and inoculated into API 20E strips, which contain 20 microtubes for biochemical reactions. Strips were incubated at 37 °C for 24 hours, and color changes were recorded after adding reagents as per the manufacturer's instructions. Numerical profiles generated from reaction patterns were compared to the API 20E database to confirm *Salmonella* identity.

Antibiotic Susceptibility Test

The antibiotic susceptibility test of the isolates was carried out as earlier described by Clinical and Laboratory Standards Institute [13]. Three (3) pure colonies of isolated bacteria species from fruits salad were inoculated into 5 ml sterile 0.85 % (w/v) NaCl (normal saline) and the turbidity of the bacteria suspension was adjusted to the turbidity equivalent to 0.5 McFarland's standard. The McFarland's standard was prepared as follows; 0.5ml of 1.172 % (w/v) $BaCl_2.2H_20$ was added into 99.5ml of 1 % (w/v) H_2SO_4.

A sterile swab stick was soaked in standardized bacteria suspension and streaked on Mueller Hilton agar plates and the antibiotic discs were aseptically placed at the centre of the plates and allowed to stand for 1 h for pre-diffusion time. The plates were incubated at 37^0C for 24 hours. The diameter zone of inhibition was measured using transparent millimeter ruler and the result of the susceptibility was interpreted in accordance with the susceptibility breakpoint earlier described by Clinical and Laboratory Standards Institute.

Classification of Antimicrobial Resistance of the Isolates

Antimicrobial resistance of the isolates was classified into: Multidrug resistance (MDR) Resistant to at least one agent in three or more antimicrobial classes; Extensive drug resistance (XDR) Resistance to all but one or two categories of antimicrobials; Pan drug resistance (PDR): Resistance to all agents in all available categories..

Determination of Multiple Antibiotic Resistance (MAR) Index

The MAR Index was determined according to the method of Krumperman 1983 as described by Mohammed *et al.* [14]. From the results of the antimicrobial susceptibility testing, MARI was calculated using the following formula:

$$MARI = \frac{a}{b}$$

Where a = number of antibiotics to which an isolate is resistant to

b = number of antibiotics against which the isolates were tested.

An isolate with MARI ≥ 0.2 is regarded to have originated from an environment where antibiotics are frequently used.

Molecular Detection of *Salmonella* sp Virulence Genes

Molecular detection of virulence genes in *Salmonella* sp is crucial for evaluating the pathogenic potential of isolates obtained from ready-to-eat (RTE) fruit salads. In this study, four virulence-associated genes were targeted: lpfC, linked to fimbrial adhesion and intestinal colonization; spvB**,** a plasmid-mediated factor involved in systemic infection; sitC, associated with iron uptake and bacterial survival; and spiA**,** a component of the type III secretion system (T3SS) essential for intracellular survival and host manipulation Haslida *et al.* [15].

Multiplex polymerase chain reaction (PCR) was employed for simultaneous amplification of these genes, using primer sets previously described by Haslinda *et al.* [15]. Reaction conditions were optimized to enhance sensitivity and specificity, while safer visualization methods were applied to reduce laboratory hazards. To assess the genetic diversity among isolates, **ERIC-PCR** (Enterobacterial Repetitive Intergenic Consensus-PCR) and **BOX-PCR** were performed, both of which demonstrated high discriminatory power in differentiating *Salmonella* strains.

In addition, statistical analyses were used to correlate the prevalence of virulence genes with antimicrobial resistance (AMR) profiles. This integrated approach provided valuable insights into the interplay between virulence and resistance in *Salmonella* serovars, contributing to risk assessment and public health surveillance of foodborne pathogens in North Central Nigeria.

DNA Extraction

DNA extraction was performed by boiling method as described by Bilung *et al.* [16]. This method of extraction was used because it is simple, fast and cost-effective way to break open cells and release DNA. Following purification on MacConkey agar, bacterial DNA was isolated from a 24 hours culture in Luria-Bertani broth prepared according to the manufacturers' protocol. The bacterial cells were harvested by centrifugation at 3200 rpm in a micro centrifuge for 2 min at room temperature and the supernatant was discarded. The pelleted cells were re-suspended in 1ml of sterile normal saline and the micro-centrifuge tubes were placed in the vortex for five seconds. Centrifugation was carried out at 3200 rpm for 1 min and the supernatant was discarded. A 0.5 ml of sterile normal saline was added to the pellets and the tubes were vortex for 5 seconds after which they it was heated in the block heater at 90°C for 10 min. immediately after heating, rapid cooling was done by transferring the tubes into the freezer at -4 temperature for 10 min. Cell debris were removed after centrifugation was done at 3200 rpm for 1 min and 300 µl of the supernatant was transferred into a steriled 2 ml Eppendof tube as DNA and stored at -10 °C until use. Estimation of the concentration, purity and yield of the DNA sample were accessed using absorbance method (measurement of absorbance) with the spectrophotometer (Nanodrop 1000). For DNA concentration, absorbance readings were performed at 260 nm (A_{260}) and the readings were observed to be within the instrument's linear range (0.1 – 1.0). Deoxyribonucleic acid (DNA) purity was estimated by calculating the A_{260}/A_{280} ratio and this was done by the spectrophotometer's computer software (where A_{260}/A_{280} ratio ranges from 1.7 – 1.9).

Amplification of *Salmonella species* virulence genes

The condition for this method was in accordance with Bilung *et al.* [16] with modifications on the reagent concentration and reaction condition. In PCR, the primer pairs that were used were ERIC 1R (5′-ATGTAAGCTCCTGGGGATTCAC-3′) and ERIC 2 (5′-AAGTAAGTGACTGGGGTGAGCG-3′). To prepare 25 µl of PCR mixture, 1.0 µM of ERIC 1R primer, 1.0 µM of ERIC 2 primer, 2.0 µl of DNA template, 1X PCR buffer, 0.2 mM dNTP, 2.5 mM MgCl2, and 2.0 unit of Taq DNA polymerase was mixed together. In BOX-PCR, the primer to be used is BOX A 1R (5'-CTACGGCAAGGCGACGCTGACG-3'). To prepare 25 µl of PCR

mixture, 0.4 µM of BOX A1R primer, 2.0 µl of DNA template, 1X PCR buffer, 0.2 mM dNTP, 2.0 mM MgCl2 and 2.0 unit of Taq DNA polymerase were mixed together. The amplification reactions for ERIC and BOX-PCR were carried out in a thermal cycler according to the condition presented in Table 1 respectively. The PCR products from ERIC- and BOX-PCR was separated in 1% agarose gel with 1 kb DNA ladder and viewed under a luminescent image analyzer (LAS-4000, FUJIFILM, Japan)

Agarose Gel Electrophoresis

The agarose gel electrophoretic assay for detection of amplified genes for different virulence genes was carried out as described by Bilung *et al.* [16]. Eight microlitre (8µl) of PCR products stained with ethidium bromide was loaded into 1.0% (wt/vol) agarose gel wells with a molecular marker operating at the same time at 120 V for 30 min. The DNA bands were visualized and photographed under UV light 595 nm. The banding patterns generated were analyzed using PyElph 1.4 software program. The dendrograms were constructed using an unweighted pair group method with arithmetic mean (UPGMA)

DNA quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was lunched by double clicking on the Nanodrop icon. The equipment was initialized with 2µl of sterile distilled water and blanked using normal saline. Two microliters of the extracted DNA were loaded onto the lower Pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the "measure" button.

16S rRNA Amplification

The 16s rRNA region of the rRNA genes of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on ABI 9700 Applied Biosystems thermal cycler at a final volume of 25 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95°C for 5 minutes; denaturation, 95°C for 30 seconds; annealing, 52°C for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72°C for 5 minutes.

Sequencing bacteria isolates DNA

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10 ul, the components included 0.25µl BigDye® terminator v1.1/v3.1, 2.25 µl of 5 x BigDye sequencing buffer, 10 uM Primer PCR primer and 2-10 ng PCR template per 100 bp. The sequencing condition were as follows 32 cycles of 96°C for 10 seconds, 55 °C for 5 seconds and 60 °C for 4 minutes.

Phylogenetic Analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using ClustalX. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 [16]. The bootstrap consensus tree inferred from 500 replicates was taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method

Statistical Analysis

Statistical analyses were carried out using SPSS version 21.0. Differences by the chi-square (χ^2) test was considered significant, if $P< 0.05$ (CI)

RESULTS

Morphological Identification of *Salmonella species* Isolated from Ready to Eat Fruit Salads

The morphological characteristics of *Salmonella species* isolates from Ready to eat Fruit Salads Sold in Plateau and Kogi in North Central States in Nigeria colonies with colorless on Xylose-lysine deoxycholate (XLD) agar and on *Salmonella-Shigella* agar (SSA) with back spot and black metallic sheen on Bismute sulphite (BSA) were *Salmonella species* grow in unequal shape, not smooth in surface, moderate in size, convex in edge, translucent and Gram negative rod shape as shown in Table 1

Occurrence of *Salmonella species* from ready to eat fruit salad

The overall occurrence of *Salmonella* species isolated from sliced mixed fruit salads 12 (4.0 %) as given in Table 2. The highest percentage occurrence of *Salmonella* species isolated from ready to eat mixed fruit salads sold in different states was from Plateau (8.0%) and the lowest was from Kogi (4.0%)

Table 1: Cultural, Morphological and Biochemical characteristics of *Salmonella*

Cultural Characteristics	Morphological Characteristics		Biochemical Characteristics								Sugar Fermentation			Inference
	Gram stain	Mopholology	Ur	Ox	MR	In	VP	it	Nit	Glu	Mal	Suc	Xyl	
Colourless colonies on SSA and black metallic sheen on BSA	-	rod shape	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	*Salmonella serovars*

DCA=Deoxycholate agar; BSA= Bismuth sulphide agar; -=Negative; +=positive; Oxd=Oxidase; Ur=Urease; Ct=Citrate; Mr=Methyl red; VP=Voges-Proskauer; Glu=Glucose; Lac=Lactose; Mal=Maltose; Mann=Mannitol; Suc=Sucrose; Xyl=Xylene

Table 2: Percentage Occurrence of *Salmonella species* Isolated from Ready to eat Fruit Salads Sold in Plateau and Kogi in North Central States in Nigeria

Location	**No. sample**	**No. isolated**	**Percentage**
Plateau	100	8	8.0
Kogi	100	4	4.0
Total	300	12	4.0

Antibiotics resistance pattern of *Salmonella serovars* isolated from ready to eat fruit salads

The antibiotics resistance pattern of *Salmonella species* isolated from Ready to eat Fruit Salads Sold in Plateau and Kogi in North Central States in Nigeria is as shown in Table 3. The order of percentage resistance of *Salmonella species* isolated from ready to eat fruit salads sold in Plateau state to antibiotics tested were cefexime (50.0 %), ofloxacin and nalidixic acid (37.5 %), ceftriaxone (25.0%), gentamicin and amoxicillin/ clavulanic acid (12.5 %). From Kogi the order of percentage resistance of *Salmonella species* to the antibiotics tested were, nalidixic acid (75.0 %), ampicillin and cefexime (50.0 %), Cefuroxime, cefotaxime and amoxicillin/ clavulanic acid (25.0 %).

Multiple antibiotics resistance (MAR) index

The MAR index of *Salmonella species* isolated from Ready to eat Fruit Salads Sold in Plateau and Kogi in North Central States in Nigeria is as shown in Table 4. The *Salmonella species* isolated with MAR index of greater than or less than 0.2 are MAR isolates and the commonest MAR in *Salmonella speciers* isolated from Plateau, the commonest MAR in *Salmonella sppecies* isolated was 0.4 with percentage of occurrence of 37.5 %. The commonest MAR in *Salmonella species* isolated from Kogi was 0.3 with percentage of occurrence of 50.0 %.

Genotypic detection of virulence genes

The result of detection of virulence genes in *Salmonella* species isolated from Ready to eat Fruit Salads Sold in Plateau and Kogi in North Central States in Nigeria is as shown in Table 5. Out of nine isolates evaluated for virulence genes, 33.3 % of the isolates harbour virulence genes. The order of virulence genes detected were, *spiA* genes (33.3 %). From *Salmonella* species isolated from Plateau harbors *spiA* (66.6 %). From *Salmonella* species isolated from Kogi harbors *spiA* (33.3 %). Plate 1 and 2 shows the molecular bands and weight of the virulence genes detected from *Salmonella* species isolated.

Table 3: Antibiotics Resistance of *Salmonella species* Isolated from Ready to eat Fruit Salads Sold in Plateau and Kogi in North Central States in Nigeria

		No. (%) Resistance *Salmonella serovars*	
Antibiotics	**Disc Content (µg)**	**Plateau (n=8)**	**Kogi (n= 4)**
Imipenem/cilastalin	30	1(12.5)	0(0.0)
Cefuroxime	30	0(0.0)	1(25.0)
Ampicillin	30	0(0.0)	2(50.0)
Nalidixic acid	30	3(37.5)	3(75.0)
Cefotaxime	30	0(0.0)	1(25.0)
Gentamicin	5	1(12.5)	0(0.0)
Ceftriaxone	10	2(25.0)	0(0.0)
Cefexime	50	4(50.0)	2(50.0)
Amoxicillin/ Clavulanic Acid	30	1(12.5)	1(25.0)
Nitrofurantoin	30	0(0.0)	0(0.0)

Table 4: Multiple Antibiotics Resistance (MAR) Index of *Salmonella species* Isolated Ready to eat Fruit Salads Sold in Plateau and Kogi in North Central States in Nigeria

			No. (%) MAR isolates	
No. of Antibiotic resistance to (a)	**No. of Antibiotic tested (b)**	**MAR (a/b)**	**Plateau (n=8)**	**Kogi (n= 4)**
6	10	0.6	1(12.5)	0(0.0)

5	10	0.5	2(25.0)	1(25.0)
4	10	0.4	3(37.5)	1(25.0)
3	10	0.3	2(25.0)	2(50.0)

Table 5: Genotypic Detection of Virulence Genes in *Salmonella species* Isolated from Ready to eat Fruit Salads Sold in Plateau and Kogi in North Central States in Nigeria

		No. (%) virulence genes			
Location	**No. of resistant**	***spvB***	***spiA***	***lpfC***	***sitC***
Plateau	3	0(0.0)	2(66.6)	0(0.0)	0(0.0)
Kogi	3	0(0.0)	1(33.3)	0(0.0)	0(0.0)
Total	9	0 (0.0)	3(33.3)	0(0.0)	0(0.0)

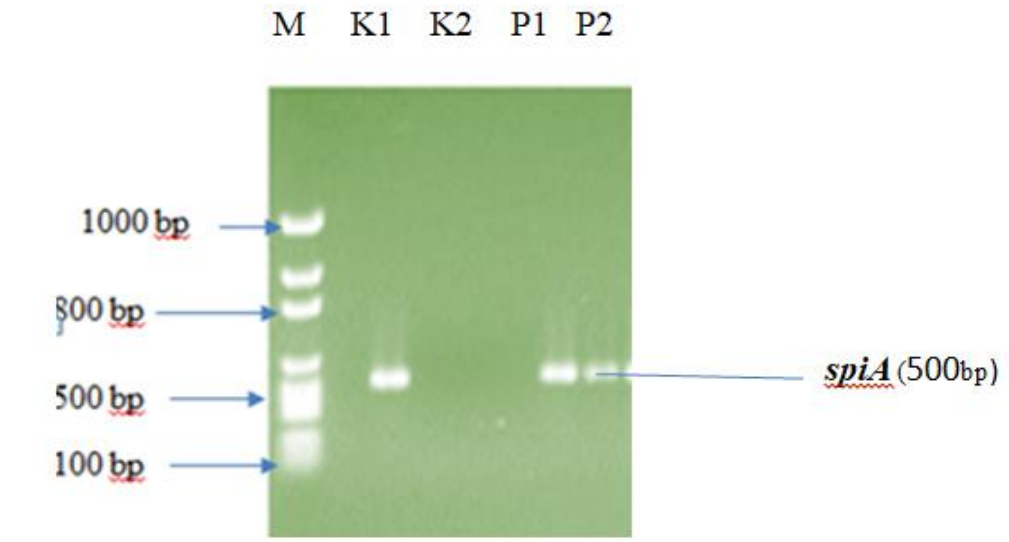

Plate 1: Agarose gel electrophoresis of the amplicons of virulence genes expression in the *Salmonella* species isolated. Lane M represents the 1000 bp DNA molecular ladder. Lane K1, p1 and p2 represent the expression of the *spiA* (500bp)

DISCUSSION

The severity of *Salmonella* infections in humans varies depending on the serotype involved and the health status of the human host. Almost all strains of *Salmonella* are pathogenic as they have the ability to invade, replicate and survive in human host cells, resulting in potentially fatal disease. *Salmonella* displays a remarkable characteristic during its invasion of non-phagocytic human host cells [17], whereby it actually induces its own phagocytosis in order to gain access to the host cell. The consumption of fruit salads over the decades has increased because they are important source of vitamins, nutrients and fiber [18], but food born disease outbreaks have been linked to pathogen contamination of sliced fruit or mixed fruit salads. In this study the percentage of *Salmonella* Sp isolated were low 4.0 % to compare to 12.1 % that was reported by Eni *et al*. [19] in Sango Ota, Nigeria. Also, it was observed that the occurrence of *Salmonella* sp from Plateau stat were higher than Kogi state with percentage of 8.0 %. The contamination of fruit salads may from equipment that are use doing the preparation of these ready to eat fruits or from the packaging materials as these fruits are cut, washed, wrapped with transparent polyethene bags before they are being sold to the consumers [20]. The rate of occurrence of

Salmonella sp in this study is similar to study reported by Oranusi and Olorunfemi [21], the low occurrence of *Salmonella* sp in the study area was not supervised due to nature of processing the ready to eat mixed fruits, the water used in clearing the fruits and improve personal hygiene of the personnel's that prepare the ready to eat fruit salads these may lead to less contamination of the ready to eat fruit. The contamination of the ready to eat fruit as observed in this study could have occurred as faecal contamination water or other materials used in processing the mixed salad and environmental factors such as dust from air [22].

The low antibiotics resistance of *Salmonella* sp to ciprofloxacin, nitrofurantoin, ampicillin and gentamicin as observed in this study was not expected because of high resistance of *Salmonella* spp that have been reported by different authors namely Adeoti *et al.* [23] reported high resistance to bacteria isolated from ready-to-eat vended fruits in Sango open-market, Saki, Oyo State, Nigeria. The findings of the antibiotic susceptibility justified the need to use of broad-spectrum antibiotics from treatment of Gram-negative bacteria infection such as *Salmonella* sp. The low antibiotic resistance may be due to the high cost of these antibiotics and the form like the gentamicin which is in an injectable and the discomfort of gentamicin injection when administered, it is likely that these antibiotics may not have been abused [24]. The amazing observation here is that the *Salmonella* sp were highly resistance to streptomycin and sulphamethoxazole/trimethoprim and this is in agreement to a study earlier reported by Mohammed *et al.* [14]. The low resistance of *Salmonella* serovars to ciprofloxacin and nitrofurantoin justifies the use of both antibiotics as drug of choice of treatment of infection cause by *Salmonella* sp.

The detection of virulence genes *spiA* genes in *Salmonella* sp isolated and this seems to disagree with other studies that show detection of more virulence genes such as *invA,* and *orgA* as reported by Haslinda *et al.* [15]. The low detection of this virulence genes mentioned above as recorded in this study may be responsible for the low or high susceptibility observed in this study, as the enzymes that inactivates the drugs were not available, although the mechanism of resistance of the antibiotics to *Salmonella* sp were not evaluated or studied in this study. The absence of most of the virulence genes may be why people that consume contaminated ready to eat fruit salads in the study area hardly come down with infection normally caused by virulence *Salmonella* species.

CONCLUSION

The isolation rate of *Salmonella* sp from mixed fruit salads was low in the study area. The isolation rate shows that most of these ready to eat fruit salads may have public health implications. Also, the *Salmonella* sp isolated from some selected market and cities in some North central states in Nigeria were susceptible to ciprofloxacin, nitrofurantoin, ampicillin and gentamicin. The virulence genes detected was *spiA*

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflicts of interest.

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