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CHARACTERISATION OF MULTI-DRUG RESISTANT *Salmonella* spp. ISOLATED FROM READY TO EAT VEGETABLE SALADS USING THE 16S rRNA SEQUENCE ANALYSIS

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ABSTRACT
Multi-drug resistance among food-borne pathogens has become a serious cause for concern and vegetable salads have been identified as a source of such food-borne pathogens. Characterization of these pathogens beyond its species level, have been known to be of very significant importance in understanding their pathogenicity. This research work was designed to molecularly characterize phenotypically identified multi-drug resistant *Salmonella* isolates from ready to eat vegetable Salads using the 16S rRNA sequence analysis. This is with a view to identifying them to sub-species level. A total of 135 samples of ready to eat vegetable salads were randomly purchased from local fast food centres, markets and roadside sellers within Benin metropolis, between October, 2016 and January, 2017. All samples collected were analyzed using standard microbiological procedures. Antibiotic susceptibility test was done on all phenotypically identified isolates after which those *Salmonella* sp. isolates showing multiple antibiotic resistance were selected and further characterized using the 16S rRNA sequence analysis. Result of the total aerobic count shows varying degree of microbial contamination ranging between 4.7×10^{3}cfu/g recorded in fast food eateries and 29.0×10^{3}cfu/g recorded from those sold in the open markets. Prevalence rates based on phenotypic identification shows that market samples had the highest rate, followed by those sold on the road side. None was recorded from any of the eateries sampled. Results of the antibiotic sensitivity test showed varying degrees of resistance to the antibiotics used in the screening with 16 of the isolates having multidrug resistance. All the isolates were resistant to amoxicillin and augmentin. Confirmatory test using the 16S rRNA sequence analysis revealed the presence of *Salmonella* spp. such as *Salmonella bongori, Salmonella enterica, Salmonella typhi* and *Salmonella typhimurium*. The result from this investigation is a pointer to the need for proper monitoring and regulation by appropriate agencies of antibiotics use and the preparation and sale of such ready to eat food in Nigerian.

KEYWORDS: Vegetable salads, *Salmonella* sp, Antibiotic Resistance, 16S rRNA sequencing

INTRODUCTION

Salad is a term broadly applied to many food preparations that have mixture of chopped or sliced ingredients which may be mostly fruits or vegetables (Uzeh *et al*., 2009). Ready-to-eat salads can be referred to as mixed fruits and vegetables that have been washed, maybe peeled, then sliced, chopped or shredded, or grated and packaged ready to be eaten without further processing.

In view of its high nutritive qualities and the ease with which it can be acquired and eaten, ready to eat salads have become very popular as they are now being sold at various points and food centres such as restaurants, fast foods places, hotels, markets and roadsides in Benin metropolis. There is actually a growing market in the sale of these ready to eat salad products which are sold in various forms in Nigeria. Some prepare and sell it under refrigerated conditions from chilled cabinets, some are acidified by the addition of mayonnaise or vinegar-based dressings, while others prepare the vegetables without any acidified dressing before packaging for sale. Whatever the form they are prepared and packaged for sale, vegetables and vegetable salads have been identified as a veritable source of pathogenic microorganisms by various authors (Halablab *et al*., 2011; Omoruyi *et al*., 2011; Mensah *et al*., 2002). Most of the outbreaks linked to these vegetables and vegetable products have been ascribed basically to various reasons such as use of unsafe water for rinsing the vegetables and sprinkling to keep it fresh, soil, faeces, animals and improper handling of the product during harvesting, transporting and processing of the vegetables into the different end products before consumption.

Bacteria of the genus *Salmonella* are widespread and are one of the most important causes of food borne infections in man. They are among the most frequently isolated etiologic bacterial agents of food borne disease outbreaks. Taxonomically, Grimont and Weill, (2007), indicated that the genus *Salmonella* consists of only two major species: *Salmonella enterica* and *Salmonella bongori*. This was also affirmed by Procop *et al.* (2017) who noted that there are two species of salmonella: *S enterica,* which encompasses six sub species and *S. bongori*. Serotypes Typhi (*Salmonella typhi*) and *Salmonella paratyphi* are highly adapted to man and have humans as one of their main reservoir with enteric fever (typhoid and paratyphoid fever) as their most important clinical manifestation. Eng *et al*. (2015) report indicates that although the incidence of typhoid fever has declined in developed countries, sporadic outbreaks continue to occur in some developed countries of the world. Enteric fever however continues to be an important cause of morbidity and mortality in developing countries where the bacterium is known to be involved in large scale food borne outbreaks causing symptoms of gastroenteritis (Andoh *et al.,* 2017). The outbreaks vary in size from a few persons affected to many thousands. Research is beginning to reveal complex relationships between microbes and plants, which seem to play an important role in the contamination process (Wogu *et al*., 2011).

The problem of multi drug resistance among bacteria organisms has become a worrisome phenomenon worldwide and in Nigeria incidences of this phenomenon have been recorded by several authors (Hart and Kariuki, 1998; Okeke *et al.,* 1999). The effect of drug resistance is such that treatment of infections become difficult because food borne organisms such as salmonella no longer respond to the commonly used drugs for treating patience that come up with the infection. The high prevalence of antibiotic resistance in bacteria in Nigeria and other developing countries has basically been ascribed to several factors including indiscriminate use due to unregulated access of non-professional to different classes of antimicrobial over-the-counter (Olatoye, 2010). For bacterial identification, 16S rRNA sequencing is becoming very important, as an improvement over the traditional methods of culture and biochemical test, which can be time consuming and sometimes fail to identify organisms to their strain levels. It has helped in providing insights into infectious diseases, antibiotics required in control of diseases and accurate identification of bacterial isolates (Drancourt *et al*., 2000).

This study is aimed at providing more information on the prevalence of food borne pathogens, such as *Salmonella* sp. and their transmission in ready-to-eat vegetable salads sold in different parts of Benin City, and the need to characterize the isolates beyond species level using 16S rRNA sequencing methods.

MATERIALS AND METHODS

Sample Collection

Samples of ready to eat vegetable salad were purchased from three each of local fast food centres, markets and roadside sellers within Benin metropolis, between October, 2016 and January, 2017. All samples were collected in duplicates in sterile containers and taken to the laboratory for immediate microbiological analysis. Fifteen samples each were collected from nine locations giving a total of 135 samples in all.

Isolation and identification of Salmonella sp

Each of the salad samples were homogenized in a sterile mortar and 1 g of the homogenate sample was suspended in 9ml buffered peptone water. Serial dilutions of up to 10^{-5} were then made and 1 ml sample of the dilution was spread using sterile glass rod, unto already prepared Nutrient Agar for total aerobic counts and Salmonella- Shigella Agar(SSA), a differential media for *Salmonella* and *Shigella* species. The plates were then incubated at 37°C for 24 hours. After the incubation time, pure cultures of all colourless colonies which appear with black centres on SSA, typical of *Salmonella* sp. were then made in readiness for biochemical tests. Biochemical tests were done according to methods outline by Holt *et al*. (1994). Isolates not used immediately were sub-cultured on agar slants and kept in the refrigerator until needed for use

Antibiotic Sensitivity Test

Antibiotics susceptibility test was performed using the disc diffusion method. The isolates were inoculated into sterile nutrient broth in test tubes and incubated for 24hours at 37°C. After 24hours isolates were streaked on already solidified Mueller Hinton agar plates. Antibiotics disk were immediately placed on the streaked agar plates with sterile forceps which were then incubated for 24hours. The antibiotics multidisc (Abtek Biologicals Ltd) used contained Cotrimoxazole (COT) 30ug, Amoxicillin (AMX) 30ug, Gentamycin (GEN) 10ug, Tetracycline (TET) 30 ug, Ofloxacin (OFL) 5ug, Augmentin (Aug) 30ug, Nalidixic acid (NAL) 10ug and Nitrofurantoin (NIT) 5ug . After incubation the zones of inhibition (in mm) around each disk was measured and interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines Interpretation of zones of inhibition are >18mm (sensitive), 13-17mm (intermediate), < 13mm (resistant) (CLSI, 2015).

Sequence Analysis

Sixteen presumptive positive isolates based on biochemical tests that were found to be multidrug resistant (resistant to three or more antibiotics) were selected and further characterized using the 16S rRNA sequence analysis.

DNA Extraction

DNA was extracted using the QIA amp DNA Mini Kit (250) cat no 51306. Buffer AE was placed into 70°C water bath. Then, 180µl of ATL was added to the isolates, 20µl of proteinase K was added and then samples were incubated at 56°C until completely lysed. The tubes were centrifuged as to collect condensation, 200µl of Buffer AL was added and vortexed for 15 seconds. The tubes were then incubated at 70°C for 10minutes after which they were centrifuged to collect condensation. 230µl of ethanol (96-100%) was added and mixed by vortexing for 30secs. Then, the samples were carefully applied to QIA amp spin column and Centrifuged at 6000g for 1minutes. The spin column was placed in a clean 2ml collection tube and the filtrates discarded. Then 500µl of Buffer AW1 was added and then centrifuged at 6000g for 1minutes and placed in the collection tube and filtrate discarded. 500µl of Buffer AW2 was added. It was centrifuged again for 1minute placed into the collection tube and filtrate discarded again. The tubes were then centrifuged at full speed for 3 minutes, column placed in labelled 1.5ml tube and 200µl of the preheated (70°C) buffer AE was added. The tubes were incubated at (70°C) for 5 minutes and then centrifuged at 6000g rpm for 1 minute, the filtrate solution (200µl) was placed back into the spin column. Then, 200µl of the preheated (70°C) buffer AE and the tubes were incubate at (70°C) for 5minutes and then Centrifuge at 6000g rpm for 1minute. Afterwards, the spin columns were discarded and ran on agarose to visualize the DNA of isolates. All molecular analysis was done at Bioscience laboratory of the International Institute of Tropical Agriculture (IITA) Ibadan, Oyo State, Nigeria.

Polymerase Chain Reaction (PCR)

Extracted DNA templates were subjected to PCR using set of primers (Forward {F} and Reverse {R}) targeting the 16S rRNA of isolates. Primer sequence is given as F-AGAGTTTGATCMTGGCTCAG and R-AAGGAGGTGWTCCARCCGCA. Thermocycling conditions used was rapid thermal ramp to 94°C for 5 minute for initial denaturation, then denature at 94°C for 30 seconds and annealing at 56°C for 30 seconds, and extension at 72°C for 45 seconds. Final extension was done at 72°C for 7 minutes; hold temperature was at 10°C. This was repeated for 36 circles with each of rapid thermal ramp to 96 °C, 96 °C for 10 seconds, rapid thermal ramp to 50 °C, 50 °C for 5 seconds, and rapid thermal ramp to 60 °C, 60°C for 4 minutes and finally rapid thermal ramp to 4 °C until ready for purification.

PCR Product Purification/16S rRNA Amplification

Absolute ethanol (20µl) was added to PCR product and incubated at room temperature after which it was spun down at 10000rpm for 15 minutes. The supernatant was decanted and span down again at 10000rpm for 15minutes then 40µl of 70% ethanol was added and the supernatant decanted again. It was then allowed to air dry and about 10µl of ultrapure water added and the product was checked in 1.5% agarose. The purified PCR product was used for another PCR reaction which then became the amplicon used for sequencing reaction.

Purifying Extension Products

The 96-well reaction plate was removed from the thermal cycler and briefly centrifuged. 5µl of 125 mm EDTA was added to each well. Then 60 µl of 100% ethanol too was also added to each well. The plate were sealed with aluminum tape and mixed by inverting 4 times and incubated at room temperature for 15 min, then centrifuged at 1650xg for 45 min at 4°C. The plates were inverted and centrifuged at 185xg then 60µl of 70% ethanol was added to each well, centrifuged at 1650xg for 15 min at 4°C. The plates were then inverted and centrifuged at 185xg for 1minute then the samples were re-suspended in injection buffer**.** The product from the purification process was loaded on the 3130xl genetic analyzer (Applied Biosystems) to give the sequences. MEGA6 was used to view and analyze the obtained data.

Generating Consensus Sequence

The bases were edited with MEGA6 software after which the reverse sequence in each case was made to complement the forward sequence by reverse complement. A pair wise alignment was carried out on both forward and reverse sequence, and the consensus sequence was obtained from the aligned sequence. The consensus sequence was pasted on blast at The National Center for Biotechnology Information (NCBI), to obtain closely related strains. The sequence data was sent to the Gen bank in Bethesda, Maryland USA, to obtain ascension numbers for each of the isolates.

RESULTS AND DISCUSSIONS

The relative increase in the consumption of ready to eat vegetable salads sold in fast food centres, open markets as well as on the road sides, in Benin City, and the observed increase in the number of persons with typhoid fever necessitated the need to investigate the prevalence rate of *Salmonella* sp. in vegetable salad. Most ready to eat foods have been confirmed to carry some form of microbial association (Adams *et al*., 1989; Adams and Moss, 2000). More so characterization of microorganisms beyond species level has become very important in view of evidential existence of various sub strains of organisms such as *Salmonella* sp. and *E. coli* implicated in various food borne disease outbreaks (Rangel *et al*., 2005; Ford *et al*., 2014).

Microorganisms affecting food come from natural micro flora or may be introduced by manufacturing and post manufacturing steps ranging from harvesting, processing, storage, distribution and sale of the food product (Enabulele and Uraih, 2009). The distribution and sale of ready to eat salad therefore becomes a suspect for the emergence of food borne disease. This study revealed that ready to eat salad samples purchased from some of the locations had significant level of *Salmonella* species isolated from them. The levels of contamination with microorganisms as indicated by the total aerobic count is shown in table 1. The results shows that those sold in the open market and road side were the most contaminated, while those sold in the regulated eateries were least contaminated with a total mean count of 15.35 x 10^3 ± 7.66 cfu/g . The result of the *Salmonella* count on salmonella-shigella agar as shown in table 2, reveal no presence of salmonella in the samples collected from the eateries, however there was some appreciable count recorded from those sampled from road side(1.74 x10^3 ± 0.24 cfu/g) and market(3.17 x 10^3 ± 0.61 cfu/g).

Based on phenotypic biochemical tests, the presumptive positive isolates of *Salmonella* sp., from different locations as shown in table 3 indicate that the Market (97.78%) and road samples (57.78%) had varying degree of Salmonella prevalence whereas none of the organisms was isolated from the eateries sampled. The presence of this enteric organism in ready to eat vegetable salad especially in these location is of public health significance as most residents buy this important ready to eat food mostly from these two sources because they are relatively cheaper and easily available.

The non-presence of *Salmonella* sp, from fast food centres in this study may be attributed to the hygienic environment observed in processing and sale point. It was observed that the eateries wash their fruits and vegetables with salt water and vinegar. We could not physically ascertain the sanitary conditions of the processing environments of salads sold in the open market and road sides as we were not able to access their processing locations. It has been previously observed by Ofor *et al*., (2009) and Osamwonyi *et al.,* (2013) that factors which could influence the proliferation of *Salmonella* sp. in ready to eat vegetable salads include the hygiene of the food handlers involved in the preparation and sale, the utensils and equipment used, mode of storage as well as sales environment. It has also been observed that most of the vegetables in Nigeria are grown with irrigated water that are in contact with grazing cattle and this can be a source of contamination as reported by Enabulele and Uraih (2009). It is our opinion that vegetables purchased from the market be properly washed with portable water and food grade solutions such as vinegar before they are used for preparing such ready to eat food like salad that does not require processing with heat to eliminate pathogenic organisms before they are consumed. It was also observed that salads available in the eateries were being sold with the addition of mayonnaise or some other form of salad dressings while most of those sold in the open market and road sides were without any acidified dressings, they were simply chopped, sliced or grated and mixed together and sold in covered plastic containers. This result is an indication of the need to check the indiscriminate sale of such food products in the open without adequate regulation and control.

The result of the antibiotic sensitivity test (table 4) shows that some of the Salmonella isolates exhibited multiple drug resistance. All the isolates were found to be resistant to Augmentin and Amoxicillin, while the least resistance was recorded for Ofloxacin (15.22%). Table 5 shows result of the Multidrug Antibiotic Resistance combination pattern of the *Salmonella* Isolates with one (1) isolate showing resistance to 8 antibiotics. In all 16(17.39%) isolates were recorded to be multidrug resistant (i.e. resistant to three or more antibiotics). This result support the earlier report of Shu-Kee *et al*., 2015 who posited that there seem to be an increase in the incidence of multi-drug resistance in *Salmonella* sp. Guerra *et al.* (2002) indicated that bacteria species such as Salmonella that are intrinsically resistant to a large range of antimicrobials of therapeutic use do so by developing the ability to acquire and transfer genetic resistance markers through plasmids. Basically, salmonella is a primary inhabitant of the gastrointestinal tract and is recognized as one of the most common cause of food borne infection worldwide.

Most often food borne infections caused by salmonella may be treated with antibiotics after confirmatory tests to ascertain if they are the agents responsible for such infection. The findings in this study that quite a percentage of the isolates (table 4) were resistant to a number of the drugs is significant and worrisome. Most worrisome is the fact that all the isolates were resistant to two very important antibiotics; Augmentin and amoxicillin. These drugs are usually the antibiotics of choice in the treatment of enteric and other related infections, however the apparent indiscriminate use and the observation that antibiotic sales and use is not properly regulated in Nigeria (Arikpo *et al.,* 2011) may be among other factors responsible for the resistance phenomenon observed in this study. Antimicrobial agents are readily available to people in local drug stores without prescription (Kwaga and Adesiyu, 1984). Such practice as noted by Enabulele *et al*. (2008) has led to misuse of antibiotics with the associated high prevalence of antibiotic resistance among isolates from animal and food sources.

Identification of bacteria using Molecular technologies is known to have enabled investigators to examine both human and environmental microbiota more deeply and sensitively than culture testing has allowed (Rhoads *et al*., 2012). Clarridge (2004) noted that the 16S rRNA gene sequences allows for bacterial identification that is more robust, reproducible and accurate than that obtained by phenotypic testing. Plate 1 is the agarose gel electrophoresis showing purified DNA before sequencing. L is DNA Ladder while lanes 1 to 16 is that of the samples. The result of the 16S rRNA sequence analysis in this work as presented in table 6, shows the presence of a diverse group of *Salmonella* species such as *Salmonella enterica, Salmonella bongori, Salmonella typhi* and *Salmonella typhimurium,* in the ready to eat vegetable salads. The identification of these very important food borne pathogens up to the specie and sub species level may not have been possible with the traditional phenotypic methods of identification also deployed in this research work. Although Clarridge (2004) in his review also noted that use of the technique may be limited by high cost, requirements for great technical skills and lack of user-friendly comparative sequencing analysis software and validated databases, our experience in recent times indicate that these challenges may no longer be an excuse for not deploying the technology for identification as most laboratories in Nigeria are now acquiring it for routine diagnosis and identification of microorganisms especially when compared to other newer technologies which are more expensive and require even more advanced technical knowhow. More so the 16S rRNA gene is reported to be universal in bacteria and so relationships can be measured among all bacteria (Woese, 1987). We have observed that most laboratories in Nigeria do not necessarily identify the organisms in their diagnosis. What they do is use serological techniques to indicate the presence of the organism and report same to the physicians who then treat without proper identity of the organism hence the result of this research work is novel as it will add to the data set of the presence of the various serovars of the organism in food sources such as salad. The presence of pathogenic and opportunistic bacteria like *Salmonella* sp in vegetable salads is of great public health concern, as most persons infected with *Salmonella* develop diarrhea, fever, and abdominal cramps 12 to 72 hours after infection. The illness usually lasts 4 to 7 days, and most persons recover without treatment. However, in some persons, the diarrhea may be so severe that the patient needs to be hospitalized (Patel *et al*., 2008). This further highlights the need to safeguard the health of the consumers by carrying out good manufacturing practices.

CONCLUSION

Conclusively, the presence of *Salmonella* sp. in vegetable salads is of significant interest in perspective of its public health significance and is indicative of health risk upon consumption of these raw vegetable salads. More so multi-drug resistance in microorganism have been known to pose serious problem in the bid to eradicate and treat food borne infectious diseases. It is therefore our opinion that concerted efforts be made during preparation to prevent the entry of such organism and the food handlers, should be educated about the path of contamination and how to prevent them during preparation and sale of the salads. This study has therefore added insights to the existing knowledge on the microbial safety of vegetable salads sold in Nigeria.

Table 1: Total Aerobic Count

Sample Clusters	Counts in cfu/g X $10^3 \pm$ SD			
	Eatery	Open Market	Road Side	Overall Mean Count
Ring Road/Oba market road axis	2.53 ± 2.27	29.01 ± 13.30	16.15 ± 6.96	
New Benin/Uselu axis	4.68 ± 3.32	25.35 ± 9.39	13.06 ± 5.27	
Ikpoba hill axis	4.34 ± 3.06	25.83 ± 9.68	17.17 ± 6.94	
Mean Count	3.85 ± 0.88	26.73 ± 1.52	15.46 ± 1.60	15.35 ± 7.66

Table 2: Total *Salmonella* count on Salmonella-Shigella agar

Sample Clusters	Counts in cfu/g X $10^3 \pm$ SD			
	Eatery	Open Market	Road Side	Overall Mean Count
Ring Road/Oba market road axis	Nil	3.80 ± 1.15	2.10 ± 1.14	
New Benin/Uselu axis	Nil	3.46 ± 1.16	1.63 ± 0.78	
Ikpoba hill axis	Nil	2.26 ± 0.93	1.50 ± 0.88	
Mean Count	Nil	3.17 ± 0.61	1.74 ± 0.24	2.46 ± 0.72

Table 3: Prevalence rate of *Salmonella* sp. in ready to eat vegetable salad based on biochemical characterization

Sampling cluster	Number of samples collected per site	Number/Percentage of samples with *Salmonella* and number of *Salmonella* isolated							
		Eatery		Market		Road side		Overall prevalence based on total number samples collected	
		No./% with *Salmonella*	No. of *Salmonella* Isolated	No./% with *Salmonella*	No. of *Salmonella* Isolated	No./% with *Salmonella*	No. of *Salmonella* Isolated	No./% with *Salmonella*	No. of *Salmonella* Isolated
Ring Road/Oba market road axis	15	0 (0)	Nil	15(100)	17	10 (66.67)	15	25 (55.56)	32
New Benin/New Lagos road axis	15	0 (0)	Nil	15(100)	21	8 (53.33)	12	23 (51.11)	33
Ikpoba hill axis	15	0 (0)	Nil	14 (93.33)	18	8 (53.33)	9	22 (48.89)	27
Overall prevalence/No. of Salmonella Isolated	45	0 (0)	Nil	44(97.78)	56	26(57.78)	36	70(51.85)	92

Table 4: Antibiotic resistance pattern of *Salmonella* isolates from ready to eat vegetable salad

	Number(percentage) Susceptibility pattern of the Isolates								
	Market (n = 56)			Road Side (n = 36)			Overall Susceptibility Pattern (n = 92)		
Antibiotic	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
COT(10ug)	24 (42.86)	0 (0.0)	32 (57.14)	11 (30.55)	1 (2.78)	24(66.67)	35 (38.04)	1 (1.09)	56 (60.87)
AMX(10ug)	0 (0.0)	0 (0.0)	56 (100)	0 (0.0)	0 (0.0)	36 (100)	0 (0.0)	0 (0.0)	92 (100)
TET(30ug)	5 (8.93)	12 (21.43)	39 (69.64)	3 (8.33)	9 (25.0)	24 (66.67)	8 (8.69)	21 (22.83)	63 (68.48)
AUG(30ug)	0 (0.0)	0 (0.0)	56 (100)	0 (0.0)	0 (0.0)	36 (100)	0 (0.0)	0 (0.0)	92 (100)
OFL (5ug)	42 (75.0)	2 (3.57)	12 (21.43)	25 (69.44)	9 (25.0)	2 (5.56)	67 (72.82)	11 (11.96)	14 (15.22)
GEN(10ug)	29 (51.78)	10 (17.86)	17 (30.36)	16 (44.44)	12 (33.33)	8 (22.22)	45 (48.91)	22 (23.91)	25 (27.17)
NAL(10ug)	31(55.36)	0 (0.0)	25 (44.64)	21 (58.33)	1 (2.78)	14 (38.89)	52 (56.52)	1 (1.09)	39 (42.39)
NIT(5ug)	9 (16.07)	13 (23.21)	34 (60.71)	6 (16.67)	7 (19.44)	23 (63.89)	15 (16.30)	20 (21.74)	57 (61.96)

COT – Cotrimoxazole, AMX- Amoxicillin, TET-Tetracyclin, AUG - Augumentin, OFL – Ofloxacin, GEN - Gentamycin, NAL - Nalidixic Acid, NIT - Nitrofurantoin.

Table 5: Multidrug Antibiotic Resistance combination pattern of *Salmonella* Isolates from ready to eat vegetable salad

S/N	Combination of antibiotic	Number of Antibiotic	Number of Resistant Isolate
1	AMX, AUG, TET, COT, NIT, NAL, GEN, OFL	8	1
2	AMX, AUG, TET, COT, NIT, NAL, GEN,	7	2
3	AMX, AUG, TET, COT, NIT, NAL, OFL	7	1
4	AMX, AUG, TET, COT, NIT, GEN	6	2
5	AMX, AUG, TET, COT, NIT, NAL	6	1
6	AMX, AUG, TET, COT, NAL, OFL	6	1
7	AMX, AUG, TET, COT, NIT	5	2
8	AMX, AUG, TET, NIT	4	1
9	AMX, AUG, COT, OFL	4	1
10	AMX, AUG, TET,	3	1
11	AMX, AUG, NIT	3	1
12	AMX, AUG, NAL	3	2
Total number of Multidrug resistant Isolates			16

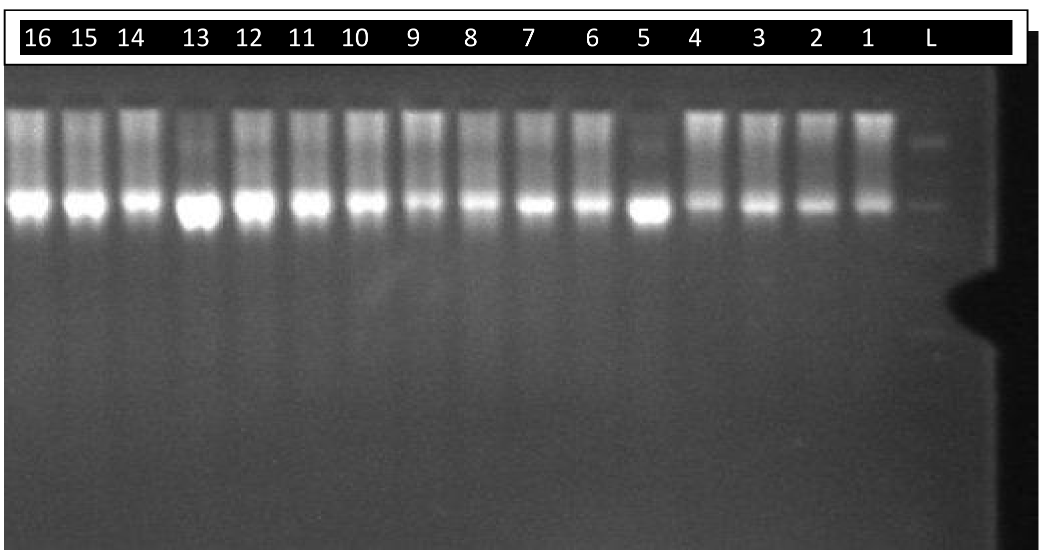

Plate 1. Agarose gel electrophoresis showing purified DNA. L is DNA Ladder while lanes 1 to 16 is samples

Table 6: Result of Sequence Analysis Producing Significant Alignment after Blast

Isolate Code	Query Cover	E Value	Identity (%)	Accession No.
1	81%	0.0	*Salmonella enterica, subsp. enterica serovar infantis*, SINFA chromosome (97%)	KY656596
2	88%	0.0	*Salmonella enterica strain* MUGA 105 16S ribosomal RNA gene, partial sequence (91%)	KY656597
3	81%	0.0	*Salmonella enterica, subsp enteric serovar*. Yovokome str. s-1850 (95%)	KY656598
4	88%	0.0	*Salmonella bongori* strain FP10 16S ribosomal RNA gene partial sequence (89%)	KY656599
5	81%	0.0	*Salmonella enterica subsp enteric serovar* Manchester str. ST278 (95%)	KY656600
6	81%	0.0	*Salmonella enterica, subsp. enterica serovar* infantis, SINFA chromosome (97%)	KY656601
7	88%	0.0	*Salmonella sp.* WZH-F23 16S ribosomal RNA gene partial sequence (96%)	KY656602
8	81%	0.0	*Salmonella enterica,* subsp. *enterica* serovar *infantis,* SINFA chromosome (92%)	KY656603
9	81%	0.0	*Salmonella enterica, subsp*. *enteric serovar Typhimurium* strain FORC_020 (87%)	KY656604
10	81%	0.0	*Salmonella typhi* strain AK-1 16S ribosomal RNA gene partial sequence (96%)	KY656605
11	88%	0.0	*Salmonella* sp. WZH-F23 16S ribosomal RNA gene, partial sequence (96%)	KY656606
12	88%	0.0	*Salmonella* sp. WZH-F23 16S ribosomal RNA gene, partial sequence (96%)	KY656607
13	88%	0.0	*Salmonella* sp. WZH-F23 16S ribosomal RNA gene, partial sequence (96%)	KY656608
14	88%	0.0	*Salmonella enterica strain* MUGA 105 16S ribosomal RNA gene, partial sequence (93%)	KY656609
15	81%	0.0	*Salmonella* sp. enrichment culture clone TB43_3 16S ribosomal RNA gene, partial sequence (94%)	KY656610
16	81%	0.0	*Salmonella enteric* subsp *enterica serovar* Manchester str. ST278 (95%)	KY656611

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