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Plasmid Profile of Antimicrobial Resistant Bacteria Isolated from Carrot (*daucus carota* l. Subsp. *Sativus*) in some selected markets in Ekiti State. Nigeria

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ABSTRACT:

Carrots are root vegetables and one of the important parts of human diet over the years. High nutrient values of the carrot (*Dascus carota* L. subsp *sativus*) and other extrinsic factors have created access for various antibiotic resistant pathogens. The aim of this study was to determine the prevalence of antibiotic resistance of bacteria isolated from carrots collected in five different locations in Ekiti-State. In this study, the microbial analysis of the carrot samples was done through poured plate method. The microbial load of the samples showed the mean bacteria count was 2.94×10^5 CFU/g and the mean total coliform count was 1.88×10^5 CFU/g, with a total of 48 bacteria isolated. Microbial characterization was carried out using cultural, biochemical and molecular techniques. The identified bacteria were *Escherichia coli* 18(37.5%), *Enterobacter aerogens* 11(20.8%), *Klebsiella pneumoniae* 8(16.7%), *Bacillus cereus* 6(14.6%), *Proteus vulgaris* 4(8.3%) and *Seretia mascescens* with 1(2.1%). Susceptibility to antibiotics was done using disc diffusion method. Plasmid detection for 15 isolates with multiple antibiotic resistances was determined by the alkaline lysis method. Plasmid curing was also done using acridine orange. In all, 41 different antibiotics resistant pattern was observed. Fifteen isolates showed multiple antibiotic resistances to at least three or more class of antibiotics. Average Percentage values of Antibiotic Resistant Bacteria ranged from 55% -100%. Percentage Antibiotic Resistant values after plasmid curing ranged from 13% -88%. In this study, the various sizes of resistant plasmids and deviation in plasmid profiles ranged from 2.4cm to 2.7cm. This study underscores the wide spread distribution of multiple antibiotic resistant bacteria in carrots and demonstrated that plasmids is one of the important ways to spread and control resistance.

Keyword: *Carrot, Antibiotic resistance, Plasmid, Chromosome, Gene.*

INTRODUCTION

Carrot, known as *Daucus carota* L. in the family of *Apiaceae* (formerly called *Umbelliferae)*, is a root vegetable with various useful bioactive nutrients such polyacetylenes, carotenoids, flavonoids, vitamins, and minerals, all of which possess numerous nutritional and health benefits to man). Carrot plant has an "umbrella-like inflorescence called an umbel" (1), a structure seen when the pedicles of each flower all diverge out of a common point on the larger stem of the plant. The most commonly cultivated variety nowadays is called *D. carota sativus.* It has an orange root and was cultivated from earlier yellow versions; yellow carrot colour is due to lutein which plays an important role in prevention of macular degeneration (the part of eye that transmit light pattern to the brain) (2,3). Besides the truth, lending the old adage that carrots are good for eyes, polyphenols, carotenoids, and vitamins present in carrot act as antioxidants, anticarcinogens (4), and immune-enhancers, Antidiabetic cholesterol and cardiovascular disease lowering, antihypertensive, hepatoprotective, renoprotective, and wound healing benefits of carrot have also been reported (5,3). The incidence of microorganisms in carrots may be expected to reflect the microbiological condition of the raw products at the time of processing. The frequent consumption of carrot is without being exposed to those processes that reliably eliminate pathogens. Human faeces are major source of many bacteria, fungi, viruses, and protozoans on carrot which can cause food poisoning. However, the nutritional value and beneficial effects to the body health had led to high consumption of carrots in recent years (6,7). There is increasing in outbreaks of human infections associated with the consumption of fresh carrots or minimally processed fruits and vegetables because various vegetables harbour a wide range of microbial contaminants which undermine their nutritional and health benefit (8). Several researches on vegetables specifically in carrots have indicated that the vegetable can be contaminated with various bacterial pathogens, including *E. coli* O157:H7, *Shigella* sp, *Listeria monocytogenes*, *Campylobacter* and *Salmonella* sp. at any of several points from the field to the time of consumption (9). Michael et al. (10) also reported *Serratia* sp. as vegetable associated bacterium which was once thought to be harmless and it is now clear that it can be opportunistic human pathogen and can infect hospitalized patients. The widespread use of various antibiotics for treating infections has created antibiotic resistant bacterial strains. Antibiotic resistance is a food safety problem for several reasons (11). However, research regarding resistance profiles of bacteria isolated from raw carrot produce is very limited. Recent research shows that antibiotic resistant bacteria also may be ingested with vegetables (12). Vegetables such as carrots, green onion and cabbage absorb antibiotics when grown in soil fertilized with livestock antibiotics contaminated manure (13). Several researches have observed antibiotic susceptibility of bacterial isolates to be dynamic and varied with time and environment. Therefore, demands need for the common bacterial pathogens isolated from carrot and carrot products to be screened periodically for their antibiotic susceptibility profiles in different cities and town in the world. According to the documentation of (14), in his findings, *E. coli* isolates in Nigeria are highly resistant to tetracycline, trimethoprim-sulfamethoxazole, amoxicillin, and ampicillin. *S. marcescens* thus demonstrates a propensity to express antimicrobial resistance. *S. marcescens* are uniformly resistant to a wide range of antibiotics including narrow-spectrum-penicillins and cephalosporins, cefuroxime, cephamycins, macrolides, tetracycline, nitrofurantoin and colistin (15). Antibiotic inactivation, Target modification, Efflux pumps and outer membrane (OM) permeability changes are the major

mechanisms bacteria use to inactivate antibiotics (16). The results of this study will provide baseline information on the: identification and characterization of bacteria isolated from carrots that are of taxonomic relevance; verification of antibiotic susceptibility of the bacterial isolates; and antibiotic resistant gene in the isolates. The aim of this study was to determine the prevalence of antibiotic resistance of bacteria isolated from carrots collected in five different locations in Ekiti-State.

MATERIALS AND METHODS

Sample Collection

Root tuber samples of carrots were collected in some randomly selected towns in Ekiti State, Southwestern, Nigeria, West Africa. Three samples each of carrot were collected from five towns namely; Ikole Ekiti, Ijesa-Isu Ekiti, Ado-Ekiti, Ode-Ekiti (Gbonyi LG), and Aramoko Ekiti (Ekiti West). The samples were collected from different hawkers in each of the towns. The samples were put in the sterile polythene bags which were properly sealed, labeled and transported on ice at 4ºC temperature to the laboratory for analyses.

Microbiological analyses

Microbiological analyses of the samples were carried out as described by (17). Biochemical tests were also performed on cultural characteristics of the isolates. These include catalase, indole, oxidase and sugar utilization (glucose, lactose and sucrose) tests using the method of Olutiola (18).

Antibiotic susceptibility testing

The Kirby- Bauer diffusion method was used to determine the antibiotic susceptibility profiles of the bacterial isolates. Pure culture of organisms were enriched and activated in 5mls of nutrient broth and incubated at 37°C to a turbidity of 0.5 Mac Farland standards, the turbidity was adjusted to match with 10^{-5}CFU/ml. The Muller Hinton (MH) agar was inoculated by streaking using sterile cotton swab of each of the cultures. After the agar surface has dried for about 5 minutes. The antibiotic disks were applied using sterile forceps and sufficiently separated from each other for about 2.5cm distance in order to prevent over lapping of the zones of inhibition. The agar plates were left on the bench for 30minutes to allow for diffusion of the antibiotic and the plates were incubated inverted at 37°C for 24hours. Results were recorded by measuring the zone of inhibition with ruler in millimeters and comparing it with Clinical and Laboratory Standards Institute (19) interpretative performance standard for antimicrobial disk susceptibility testing. The following common antibiotics were used with their inhibitory concentration: Augmentin(30µg), Cephalexin(30µg), Ceftazidime(30µg), Penicillin(10µg), Tetracycline(30µg), Ampicillin(10µg), Nitrofurantoin(30µg), and Perfloxacin(5µg).

Molecular Identification of the isolates

Extraction of bacterial DNA

For extracting bacterial genomic DNA, bacterial strain was incubated in 5 ml of medium as mentioned above and bacterial cells were collected from 1 ml of the culture by centrifugation at 13,500 rpm for 3 mins. The pellet was resuspended in 200 µl of STET buffer (0.1 M NaCl, 10mM Tris-Cl (pH 8.0), 1mM Ethylenediamineetetraacetic acid (EDTA) (pH 8.0) and 5% (v/v) Tween 20), from which the genomic DNA was extracted with a guanidium thiocyanate based method Vuong et al. (20). Finally the DNA pellet was resuspended in 200 µl of 10 mM Tris-Cl (pH 8.0), 1 mM EDTA (TE) buffer and 5 µl of the extract was run on 1% (w/v) Agarose gel to detect the presence of DNA.

Polymerase chain reaction (PCR)

About 2.5ng of bacterial genomic DNA was added to a 50 µl PCR mixture which contained 1 X Hot start reaction buffer, 0.25 mM dNTPs, 0.01 M (each) primers 27F and 1525R, and 2.5 U Hot start polymerase (Jenabioscience). Thermal cycling was done in a veriti thermal cycler (Applied Biosystems, USA) and cycling conditions were 95ºC for 3 min followed by 45ºC cycles of 95ºC for 30 secs, by 45ºC for 1 min, 72ºC for 1 min 30 secs with ramp from 45ºC to 72ºC set at 40%. Subsequently, the reaction was held at 72ºC for 10min after which it was held at 4ºC till terminated. PCR products were resolved on 1% (w/v) agarose gel stained with Ethidium bromide and viewed on a transilluminator (21).

Sequencing of amplified 16S rRNA gene

The PCR products were purified using Montage PCR Clean up kit (Millipore). The purified PCR products of approximately 1,500 bp were sequenced using 4 primers (27F - AGA GTT TGA TCM TGG CTC AG, 1492R - TAC GGY TAC CTT GTT ACG ACT T, 518F - CCA GCA GCC GCG GTA ATA CG and 800R - TAC CAG GGT ATC TAA TCC). Sequencing was performed using Big Dye terminator cycle sequencing kit v.3.1 (Applied BioSystems, USA) and sequencing products were resolved on an Applied Biosystems model 3130XL automated DNA sequencing system (Applied BioSystems, USA) at the Macrogen, Inc., Seoul, Korea, Lachance et al. (22).

Bioinformatic analysis

All sequences were compared with reference sequences in the Ribosomal Database Project (RDP) using sequence Match and the sequence were analyzed in GenBank using the BLAST (Basic Local Alignment Search Tool) bioinformatics program on the NCBI (National Center for Biotechnology Information) website. BLAST was done to identify 23S rRNA sequences in Genbank most similar to the query sequence sent.

Plasmid extraction: tens – Mini Prep (for bacteria) Protocol

A volume of 1.5ml of overnight culture was spinned for 1 minute in a micro-centrifuge to pellet cells. The supernatant was gently decanted, leaving 50-100µl together with cell pellet and vortex at high speed to re-suspend cells completely. 300µl of Tris 25mM, EDTA 10mM, NaOH 0.1N and SDS 0.5% were added and mixed by inverting tubes 3-5times until the mixture became sticky. 150µl of 3.0M Sodium acetate was added with a pH of 5.2 to mix completely. It was spinned for 5minutes in micro-centrifuge to pellet cell debris and chromosomal DNA. Supernatant was transferred into a fresh tube and mixed well with 900µl of ice-cold absolute ethanol. It was spinned for 10minutes to pellet plasmid DNA. (White pellet was observed). Supernatant was discarded and the pellet was rinsed twice with 1ml of 70% ethanol and dried. For further use, re-suspend pellet in 20-40µl of Tris 25mM, EDTA 10mM buffer or distilled water. (TENS Composition: Tris 25mM, EDTA 10mM, NaOH 0.1N and SDS 0.5%). (23,24,25).

Agarose Gel Electrophoresis

Agarose Gel Electrophoresis is a separation method that can be used to separate DNA based on their molecular weight. The concentration of Agarose used is dependent on the size of DNA to be separated but basically they can be used as follows: Plasmid DNA – 0.8% Agarose. Provision was made with the following: 1X TBE buffer (or 1X TAE Buffer) (Tris Boric acid (or Acetic acid) EDTA, Agarose powder, (λ-DNA HIND III digested), 0.5-10µl micropipette and tips, Ethidium bromide (1mg/ml), Loading dye.

Plasmid Curing

Bacteria cells were grown in nutrient or Mueller Hinton broth overnight. 5mls of Nutrient broth supplemented with 1mg/ml acridine orange was prepared, and the organisms was sub-culture into Nutrient Broth containing the acridine orange, and Incubated at 37ºC from 48 hours to about one week. Cured organism was plated out on Nutrient agar.

Plate1: shows the isolates with cured plasmids

Post-Plasmid curing

After plasmid curing, antibiotic susceptibility testing using the initial antibiotics to which organisms were resistant following the similar procedure in antibiotic susceptibility testing.

RESULTS AND DISCUSSION

Carrots are essential parts of the diet of humans and have been considering as carrier of bacteria and toxins either from the field or both processing and storing errors. Raw and uncut carrots grown in treated soil are generally considered safe for consumption, but transporting and improperly cleaning soil, water and propagated carrots can transfer dangerous bacteria to the carrots and carrot products. When carrot products canned or bottled incorrectly, they can allow for bacteria and toxin development and can cause serious illness globally. Raw carrots are widely consumed in salads, spices, fried rice and roasted meats in most countries, including Nigeria. Consumption of raw or slightly cooked carrot can increase the risk of food-borne disease. Reports showed that there was an increase in the number of outbreaks of food-borne diseases associated with consumption of fresh produce (26). In most of the reported outbreaks of gastrointestinal disease, fresh produce were found to be responsible for bacterial contamination (especially with members of *Enterobacteriaceae* family). The total plate count of all the samples analysed was presented in Table 1. The total bacteria count ranged from $1.4x10^5$ CFU/g to $2.1x10^5$ CFU/g with the mean count of 2.94×10^5 CFU/g. Also, the total coliform count ranged from $0.9x\ 10^5$ CFU/g to $4.8x10^5$ CFU/g with the mean of $1.88x10^5$ CFU/g. The count obtained from this study is lower to the one obtained by Adesetan et al. (27) who reported $3.0 – 9.3 \times 10^6$CFU/g when studied on the bacteria in fruits. Also Ogunbanwo et al. (28) also reported a high microbial load of $5.3x10^6$ CFU/ml in water melon fruits. A total of 48 bacteria species were isolated from the samples as it was shown in figure 1. Six different bacteria genera were identified. The most predominant bacteria was *Escherichia coli* 18(37.5%), this is follow by *Enterobacter aerogens* 11(20.8%), *Klebsiella pneumoniae* 8(16.7%), *Bacillus cereus* 6(14.6%), *Proteus vulgaris* 4(8.3%) and *Seretia mascescens* with 1(2.1%). This is in accordance with the work of Adebayo et al. (29) that showed *Escherichia coli* (28.6%) were most predominant bacterial isolates associated with vegetable spoilage in Uyo metropolis, followed by *Enterobacter* spp. (21.4%), *Erwinia* spp. (14.3%), *Staphylococcus aureus* (14.3%). *Bacillus cereus* and *Listeria monocytogenes* are two forms of bacteria that grow in soil, and can contaminate carrots if the soil is contaminated. *Bacillus cereus* can also produce spores and toxins, which can contaminate stored carrots prior to eating. The results of this study demonstrated that the carrot samples vended for consumers' consumptions were contaminated by pathogenic bacteria which if ingested may be deleterious to consumer's health and may lead to foodborne illness or diseases. Also, the work is also agreed with the previous work of Adesetan et al. (27) that reported *Staphylococcus aureus*, *Micrococcus* sp, *Bacillus subtilis*, *Lactobacillus* spp, *Streptococcus* spp, *E. coli*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Serratia plymuthica*, *Serratia ficaria*, *Proteus mirabilis* and *Enterococcus faecalis* as some of the bacteria that can be isolated from fruits. Also, Tambeker et al. (30) also isolated *E. coli*, *P. aeruginosa*, *Salmonella*, *Proteus*, *S. aureus*, *Klebsiella and Enterobacter* from street vended fruits juices in Amravaticity, India. There are several reports on the disease outbreaks cause by pathogenic bacteria in carrots: *K. pneumoniae* has been recognized as an important food-borne pathogen in carrot (31), meningitis, which is caused by *Serratia marcescens*, has been reported from the paediatrics wards. Pathogenic *Xanthomonas campestris* in carrot has also documented by (1). Since carrot can be ingested directly without warming or heat-treated, also take important parts of human diet and food supplements, an increased number of microbial infections associated with consumption of these carrots have been reported in recent years.

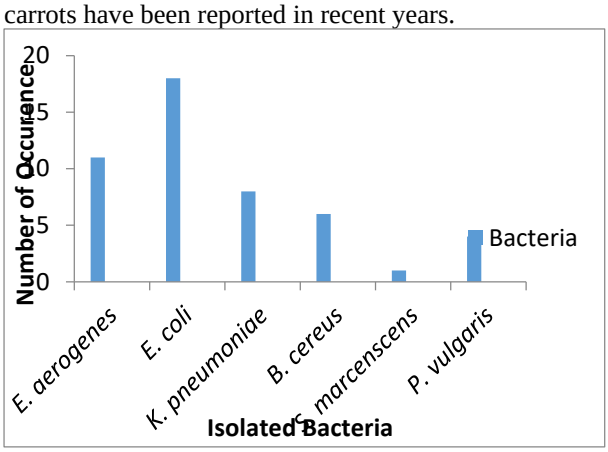

Figure 1: A bar graph illustrates occurrence of the isolated bacteria in carrot samples.

Table (2) showed that *E. coli* exhibited high percentage resistance to augmentin (89%), penicillin (83%), ampicillin (78%), cephalexin (72%), and ceftzidine (72%). Nitrofuratoin and perfloxacin both exhibited 39% showed that *E. coli* strains isolated from carrots were more susceptible to them. *K. pneumoniae* also exhibited high percentage resistance against penicillin (100%), cephalexin (88%), ceftzidine, augmentin, tetracycline, ampicillin, and nitrofuratoin, all exhibited 63%, while perfloxacin exhibited very low percentage resistance against *K. pneumoniae* with 25%. Thus *B. cereus* showed high percentage in multiple antibiotic resistance against ceftzidine, augmentin, tetracycline, ampicillin, and penicillin with 71% each, while percentage resistance of *B. cereus* to perfloxacin was very low. *E. aerogenes* exhibited high percentage resistance to augmentin (82%), ampicillin (73%), penicillin (91%), ceftazidne (64%), and tertracycline (64%). Similarly, *P. vulgaris* exhibited 100% resistance to penicillin and ampicillin, 75% resistance to tetracycline and augmentin, while *P. vulgaris* was unable to exhibit resistance to nitrofuratoin. *P. vulgaris* was also antibiotic tolerance to cephalexin (50%) and perfloxacin (50%). However, *S. marcescens* exhibited antibiotic resistance to all used antibiotics in exception of cephalexin. More so, highest percentage in Average Resistance to antibiotics was exhibited in *S. marcescens* with 100% follow by *P. vulgaris* (69%), *E. coli* (67%), *B. cereus* (57%), and *K. pneumoniae* (55%). This agreed with the study of (32) who documented in his findings that multiple resistance genes are harbored on resistance plasmids (R-plasmids), some of which are conjugative. Rasko et al. (33) reported that both pathogenic and non-pathogenic strains resistant to drugs may be transported from animals to humans

via food. Such strains act as an important source for *in vivo* transmission of R-plasmids to drug sensitive strains in the animal bowel mainly through conjugation (34). Other workers reported that transmission of resistance plasmids of *E. coli* from poultry to human commonly occurs (35).

Table 1: The total bacteria count of carrot samples collected from five different towns in Ekiti State.

Sample code	Dilution factor	TBC	TCC	TBC CFU/gX10^5	TCC CFU/gX10^5
IJ	10^{-4}	16	10	1.6	1.0
AD	10^{-4}	13	22	1.3	2.2
OD	10^{-4}	21	14	2.1	1.4
AR	10^{-4}	9	9	0.9	0.9
IK	10^{-4}	14	48	1.4	4.8
		15.6	20.6	2.94	1.88

Key: TCC; total coliform count, TBC; Total bacteria count, CFU; Colony forming unit.

Table 2: Percentage Distributions of Antibiotic Resistant Bacteria isolated from carrot samples.

Bacteria	n	AUG	CEP	CEF	PEN	TET	AMP	NIT	PER	AVR
E. coli	18	16(89)	13(72)	13(72)	15(83)	11(61)	14(78)	7(39)	7(39)	12(67)
K. pneumoniae	8	5(63)	7(88)	5(63)	8(100)	6(33)	6(33)	6(33)	2(25)	6(55)
B. cereus	6	5(71)	2(29)	5(71)	5(71)	2(29)	5(71)	4(57)	0	5(57)
E. aerogenes	11	9(82)	6(55)	7(64)	10(91)	7(64)	8(73)	3(27)	4(36)	7(62)
P. vulgaris	4	3(73)	2(50)	3(75)	4(100)	3(75)	4(100)	1(25)	2(50)	3(69)
S. marcescens	1	1(100)	0	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)

Key: AUG = Agumentin, CEP = Cephalexin, CEF = Ceftazidine, PEN = Penicillin, TET = Tetracycline, AMP = Ampicillin, NIT= Nitrofuratoin, PER = Perfloxacin, AVR = Average Resistance

Table3: Antibiotic Susceptibility Test of Resistant Bacteria after Plasmid Curing.

Bacteria	Time	Aug	Cep	Cef	Pen	Tet	Amp	Nit	Per	n(%)	Resistant Patterns
E. coli	after	S	I	S	S	R	S	S	S	1(13%)	Tet
	before	R	R	R	R	R	R	I	S	6(75%)	aug- cep- cef- pen- tet- amp
K. pneumonia	after	R	R	R	R	R	R	R	S	7(88%)	aug; cep- cef- pen- tet- amp- nit
	before	R	R	R	R	R	R	R	S	7(88%)	aug; cep- cef- pen- tet- amp- nit
P. vulgaris	after	I	R	R	I	R	R	R	S	5(63%)	cep- cef- tet- amp- nit
	before	I	R	R	R	R	R	R	S	6(75%)	cep- cef- pen- tet- amp- nit
E. coli	after	S	S	I	S	S	R	S	I	1(13%)	Amp
	before	R	I	R	R	S	R	S	R	5(63%)	aug- cef- pen- amp- per
K. pneumonia	after	S	R	S	S	S	S	R	S	2(25%)	cep- nit
	before	S	R	R	R	R	I	R	R	6(75%)	cep- cef- pen-tet- nit- per
P. vulgaris	after	R	R	R	R	S	R	S	R	6(75%)	aug- cep- cef- pen- amp- per
	before	R	R	R	R	S	R	S	R	6(75%)	aug- cep- cef- pen- amp- per
E. coli	after	R	S	S	S	S	S	S	S	(13%)	Aug
	before	R	R	S	R	S	R	R	R	6(75%)	aug- cep- pen- amp- nit-per
E. coli	after	S	S	S	S	R	S	R	S	2(25%)	tet- nit
	before	R	S	I	R	R	R	R	S	5(63%)	aug- pen- tet- amp- nit
E. coli	after	S	S	I	S	S	S	S	R	1(13%)	Per
	before	I	R	R	R	I	R	R	R	6(75%)	cep- cef-pen- amp- nit-per
E. coli	after	S	S	R	S	R	S	S	R	3(38%)	cef-tet-per
	before	R	R	R	I	R	S	S	R	5(63%)	aug- cep- cef-tet- per
Percentage Resistance	after	3(30%)	4(40%)	4(40%)	2(20%)	5(50%)	4(40%)	4(40%)	3(30%)		
	before	7(70%)	8(80%)	8(80%)	9(90%)	6(60%)	8(80%)	7(70%)	6(60%)		

Key: I = Intermediate, R = Resistant , S = Sensitive, Aug = Augmentin, Cep = Cephalexin, Cef = Ceftazidine, Pen = penicillin, Tet = Tetracycline, Amp = Ampicillin, Nit= Nitrofuratoin, Per = Perfloxacin

E. coli can also contaminate carrots during some steps in the processing. The greatest risk, it states, is during the handling, washing, grading and packing of the carrots. In addition to the risk of processing, the retailing method of carrots can pose a risk for cross-contamination, since carrots are often sold in bulk displays and could be accidentally contaminated by other individuals. In 2005, spoiled airline food caused a food-borne illness among several passengers. The food culprit was identified as carrots produced in a factory in Honolulu. While the Food and Drug Administration did not identify the factory as causing the Shigella contamination, Fox News reports that the factory had failed a food health inspection four months prior to the outbreak. According to that report by (36), the company's spokesman said the factory was cleaned, staffs were retrained, management was reorganized, and additional cleaning staffs were hired in response to the health inspection, but he contended that the factory had not been identified as the source. Fox News describes a lawsuit that blames the unsanitary factory conditions for causing the contamination of the carrots, but it does not give the conclusion. In 2007, according to the document of Kimberly (2013), again Shigella had contaminated baby carrots distributed by Kroger Company and by Trader Joe's. A product recall was initiated after the contaminants were discovered in Canada. Pritzker Law reports that four cases were reported regarding illnesses from the baby carrots, but no hospitalizations or deaths occurred from the infection. The source of the contamination is still being researched.

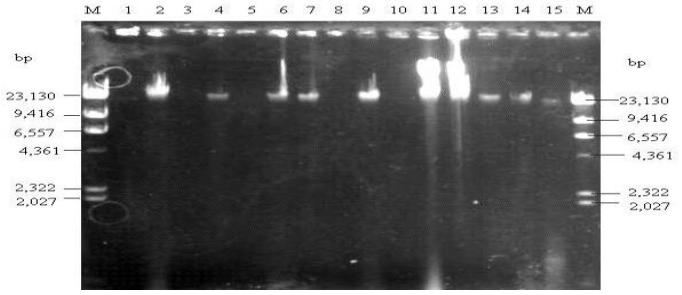

Plate 2: Agarose gel photographs of Plasmid DNA of fifteen slected multiple drug resistant bacteria.

1= *B. cereus*, 2 = *E. coli*, 3= *S. marscensen*, 4= *K. pneumoniae*, 5= *K. pneumoniae*, 6= *P. vulgaris*, 7= *E. coli*, 8= *E. coli*, 9= *K. pneumoniae*, 10= *B. cereus*, 11= *P. vulgaris*, 12= *E. coli*, 13=*E. coli*, 14=*E. coli*, 15=*E. coli.*

Plasmids are the major mechanism for the spread of antibiotic resistant genes in bacterial populations (37,38). Plate (2) shows the plasmid profile of selected fifteen bacteria species for plasmid analysis. 10 bacteria species from all the fifteen showed the present of plasmid with the same molecular weight and different sizes. Out of the 15 bacteria subjected to plasmid profile, ten isolates (isolate 2, isolate 4, isolate 6, isolate 7, isolate 9, isolate 11, isolate 12, isolate 13, isolate 14, and isolate 15) found with plasmid. Isolates 2 and 4 have the same plasmid size of 2.5cm, isolates 6 and 7 have the same plasmid size of 2.6cm, isolate 9, isolate 13 and isolate 14 have the same plasmid size of 2.7cm, isolate 11 has plasmid size of 2.4cm and isolate 12 has plasmid size of 2.3cm, with the exception of isolate 1, isolate 3, isolate 5, isolate 8 and isolate 10 which didn't carry plasmid. Resistance plasmid in molecular epidemiology of has been a major issue since investigators/scientists became aware of its role in the spread of antibiotic resistance. The plasmid replication system, which dictates the plasmid's behavior (host range, copy number) is the major plasmid landmark from a biologic standpoint; it is used for plasmid classification and identification (39). However, their number (plasmid copies) also plays a critical role in imparting various characteristics to the pathogen, such as resistance towards different antibiotics.

CONCLUSION

In conclusion, the result of this study shows that the carrot samples collected from five different markets in five-different Local Government in Ekiti State were heavily contaminated with resistant bacteria and is of special concern for human consumption. Plasmid profile study results demonstrated that resistant *K. pneumoniae* (AR10) and *P. vulgaris* to various used antibiotics is not plasmid mediated but others showed positive plasmid mediation. The results of this study demonstrated that the plasmid is one of the important ways to spread resistance but chromosomal mutation by environmental selection might also responsible for resistance.

Further research is needed to determine all major sources of antibiotic resistant food borne pathogens in carrots and carrot products. Data on the prevalence and types of antibiotic resistance in microorganisms isolated from carrots may help to explain the role of foods in the transmission of antibiotic-resistant strains to human populations.

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