DETERMINATION OF VIRULENCE GENES IN EXTENDED SPECTRUM BETA LACTAMASE-PRODUCING E.COLI ISOLATED FROM FRESH LEAFY VEGETABLES SOLD IN RIVERS STATE, NIGERIA.

Comment [WPS_165741]: Should be captured in the title of the work as Escherichia coli and in italics

Abstract :

The aim of this study is the determination of virulence genesESBL- producing *Escherichia. coli*isolated from cabbage, spinach and water leaf. Extended-spectrum β-lactamases (ESBL'S) are a rapidly growing group of β-lactamases which have the ability to hydrolyze third generation antibiotics such as Cephalosporins and Aztreonam, but are inhibited by Clavulanic Acid.The randomised experimental design was used to collect cabbage (*Brassicaoleracea*) , broad leaf water leaf (*Hydrophyllummacrophyllum*) and spinach (*Spinaciaoleracea*)samples from retailer sellers in two different markets (Choba and Rumuosi daily markets) of Obio/Akpor Local Government Area, Rivers State.Water leaf samples had a mean count of 2.9×10^3,spinach sample mean count was2.8×10^4 Cfu/g AND the highest, while the lowest mean count was obtained from cabbage samples;1.6×10^3Cfu/g.The study has established the presence of pathogenic *E.coli* (Enterohaemorrhagic) which are Shiga toxin producersTHAT AREHighly virulent with low infections doses.

Comment [WPS_165742]: Add "in" between genes and ESBL in the sentence "virulence genes "in" ESBL producing Escherichia coli

Comment [WPS_165743]: This statement should come before stating the aim of the work.

Comment [WPS_165744]: Should be Brassica oleracea

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Comment [WPS_165746]: retail sellers not retailer seller

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Keywords:virulence genes, Shiga toxin, Bacterial pathogens, antibiotics

INTRODUCTION

Bacterial pathogens of public health importance such as *Escherichia coli 0157:H7*, *Salmonella*, *Shigella*, *Staphylococcus aureus* and *Clostridium spp*. have been isolated from fresh-cut ready to eat fruits, vegetables and ready-to-eat foods sold on the streets, markets, schools, major cities and fast-food joints/restaurants in Nigeria (Ogidi*et al.*, 2016).Marnizumeet al. (2005) and Sugiyamaet al.(2005) described*Escherichia coli* as afacultative anaerobic gram-negative rod-shaped bacterium that can causes infection in both humans and animals. Doyle et al. (1989)in an earlier work reported that majority of the bacterial disease transmission occurs through eating of undercooked contaminated ground meat, consumption of raw milk, cooked and raw vegetables, fruits contaminated by water, cheese, curd and also through consumption of sprouts, lettuce and juice.

Extended-spectrum β-lactamases (ESBL'S) are a rapidly growing group of β-lactamases which have the ability to hydrolyze third generation antibiotics such as Cephalosporins and Aztreonam, but are inhibited by Clavulanic Acid.They represent the first example in which β-lactamase–mediated resistance to β-lactam antibiotics resulted from fundamental changes in the substrate spectra of the enzymes (Philipponet al., 1989). Atpresent, β-lactam drugs are a key factor in the treatment of bacterial infections worldwide and account for almost 65% of antibiotic usage (Patel, 2018). They have been classified into six main groups based on the chemical structure of the β-lactam ring which includes Penicillins, Cephalosporins, Cephamycins, Carbapenems, Monobactams, and β-lactamase inhibitors. These drugs block cell wall synthesis by preventing accurate working of the Penicillin-binding protein (PBP), which has a principal role in the synthesis of the bacterial cell wall, and finally leads to cellular death. Nevertheless, it is unfortunate that, in recent years, resistance to this important class of antibiotics is also increasing globally.ESBLs are mostly produced by gram-negative bacilli, especially *Enterobacteriaceae* family. Enterobacteriaceae, especially *Escherichia coli*,Salmonella, Shigella and *Vibriocholerae* (Pitout*et al.*, 2005). ESBLs are often encoded

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by genes located on large plasmids, and these also carry genes for resistance to other antimicrobial agents such as aminoglycosides, trimethoprim, sulphonamides, tetracyclines and chloramphenicol (Paterson, 2000).

After the widespread use of the expanded-spectrum cephalosporins,strains of*Klebsiella pneumoniae* that were resistantto these new drugs began to appear(Cohen,2000),resistance spread to some strains of*Escherichia coli* and, in rare instances, to other gram-negativeorganisms as well. Investigation into the mechanisms responsiblefor this resistance revealed the presence of new forms ofthe older plasmid-mediated TEM and SHV b-lactamases. Mutationswithin the structural genes encoding the older enzymeshad occurred, giving rise to derivatives that possessed an extended substrate profile compared with that of the parentalenzymes. Thus, these new enzymes were given thename Extended-Spectrum β-Lactamases (ESBLs) to reflect thefact that they were derivatives of older enzymes and had a newcapability to hydrolyze a broader spectrum of b-lactam drugs.the development of resistance to antimicrobial is known to occur through stable genetic change heritable from generation to generation through specific mechanisms including mutation, transduction, transformation and or conjugation (Cohen, 2000).

Escherichia coli, an important gastrointestinal flora known to be capable of accepting and transferring plasmids and which under stress readily transfers those plasmids to other species, is therefore considered an important reservoir of transferable antibiotic resistance genes ((Rahman, 2018). Investigation by CDC indicates that 10.8% of a zero type of this bacterial (e.g*E coli* 0157:H7) tested in 2006 have become resistant to one or more antibiotics (CDC,2006).Several outbreaks of *E.coli* O157, 0157:H7 and other sero type of this bacterial have been epidemiologically attributed to consumption of contaminated fresh and cooked vegetables and meat products (CDC, 2020). The aim of this study is the determination of

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Comment [WPS_1657414]: TEM and SHV should be define first before been used as abbreviation

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virulence genesESBL- producing *Escherichia. coli*isolated from cabbage, spinach and water leaf.

2. METHODOLOGY

2.1 Research Design

The randomised experimental design was used to collect cabbage (*Brassicaoleracea*) , broad leaf water leaf (*Hydrophyllummacrophyllum*) and spinach (*Spinaciaoleracea*)samples from retailer sellers in two different markets(Choba and Rumuosi daily markets) of Obio/Akpor Local Government Area, Rivers State.All samples analyzed were within a period of two weeks, the samples were held at 4^{o}c for not more than 30min before analysis. The samples were pre enriched, serially diluted and plated on a selective medium, further test was carried out on the isolated microorganisms.

2.2 collectionof samples.

A total of 30samples comprising10 for each of fresh cabbage,spinachand ten wraps of fresh water leaves were randomly boughtfrom two Choba and Rumuosi retail markets during the morning hours of the day for five days.

2.3Methods of Samples Analysis

Pre enrichment

Twenty-five grams (25gms) of each of three leaf sample was agitated differently in 225mls of 0.1% of peptone water (Oxoid UK)for 2 min to suspend surface microbes (Seow*et al.*, 2012).The homogenate was incubated at 35^{0}C for 6hrs.

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should provide detail information on how you process you samples in the laboratory up till the end of their use

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Isolation of *Escherichia coli*

Tenfold serial dilutions of the pre-enrichedleave samples using sterile 0.1% peptone water as diluent, was made after 6hrs of pre-enrichment.Aseptically 1ml of the homogenized sample was collected using a sterile pipette into 9ml of the sterile diluent which resulted into dilution 10^{-1}, from the dilution 10^{-1}, 9mls was taken into another 9ml diluent to make dilution 10^{2} this process continued until dilution 10^{-6} was obtained.0.1ml from each dilution was platedon MacConkey agar and Eosin methylene blue agar respectively andincubated at 35^{o}Cfor 24hrs.After the incubation period, the green metallic sheen colonies on Eosin Methylene Blue Agar presumptively identified as *E.coli* were collected and sub cultured for purification and stored onnutrient Agar slants for preservation.Identification of the isolates was by Grams Staining, and Biochemical Tests.

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2. 4 Antibiotic sensitivity screening of Positive isolates of *E coli*

Antibiotic sensitivity screening of test isolates was done using standard disk diffusion test method according to CLSI M100(2020).

Inoculum preparation by Direct Colony Suspension Method

Five milliliters (5mls) of sterile 0.85% normal saline was prepared; a loopful of the test isolate (18hrs) was collected and inoculated into the saline to make a suspension. The

suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland standard (suspension contains approximately 1×10^8 CFU/mL), this was performed visually by using adequate light to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black line.

Disk Diffusion Test

Fifteen minutes after adjusting the turbidity of the inoculum suspension, a sterile swab stick was dipped into the adjusted suspension, the dry surface of the Muller Hinton agar was inoculated by streaking the swab over the entire sterile agar surface this was repeated twice to ensure even distribution of the inoculum.It was left for 15mins before applying thedisc containing multipleantibiotic and incubated at 37^{o}C for 18hrs. After 18hrs, the plates were examined for inhibition and diameter of zone of inhibition measured. The measurements were translated into susceptible, intermediate, and resistant categories according to the zone interpretation table (CLSI). The presence of a growth inhibition zone larger than or equal to the established breakpoint diameter is an indication of susceptibility to that antimicrobial agent.

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2. 5Phenotypic Detection of Extended Spectrum Beta Lactamase

Kirby-Bauer disk diffusion method (CLSI M100, 2020)

Detection of ESBL-producing organisms was performed by Double Disc Synergy Test (DDST) method following CLSI recommendations. In this method, first, a suspension of each of the pure bacterial isolate was prepared according to the 0.5 McFarland turbidity standard and cultured on Mueller–Hinton agar. Fifteen minutes after bacterial cultures, pairs of antibiotic disks containing Cefazidime (30 µg) with Cefazidime/Clavulanic acid (30/10 µg), and Cefotaxime (30 µg) with Cefotaxime/Clavulanic acid (30/10 µg) were placed

on Mueller–Hinton agar medium center to center, at a distance of 20 mm apart from each other. The plates were incubated for 24 h at 37 °C. Thereafter, the diameter of inhibition zone was measured. According to CLSI, (2020) guidelines, an increase of ≥5 mm in the zone diameter around the clavulanic acid combination disks versus the same disks alone confirmed the presence of ESBL producer strains.

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2.6 Determination of e. coli Virulence Genes

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DNA Extraction

The extraction of DNA from the isolated E. coli was done using the boiling method. This was done by taking a loopful of the bacteria and suspended in 150mlof distilled water,the suspension was boiledat 100^{0}cfor 10mins in a water a bath and was placed on ice for 5mins, and centrifuged for 5minutes at 10,000g to obtain the supernatant. The supernatant was stored in an Eppendorf tube.The DNA was subjected to cocktail mix and condition for PCR. The PCR cycling for *E coli*strains consisted of 95^{0}C for 5min which was followed by 40 cycles of denaturing at 950c for30s, annealing at 600c for 30s and elongation at 720c for 30s.

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Gel electrophoresis

The presence or absence of band were assessed using gel electrophoresis with a potable gel hood built in blue LED (470nm) by Royal Biotech/Biolympics,1.5% agarose gel at a constant voltage, IX TBE for approximately 1 hour. They were visualized by ethidium bromide staining and photographed under ultraviolet light. the ladder used 100 base pair ladders fromThermofisher.All data obtained were analysed using Microsoft Excel

4. RESULTS

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thevarious results obtained from the study are presented in the tables and figures listed below:

Table.1: Total green metallic sheen colony counts OF the three samples from the different locations

Sample	CFU/G	LOG CFU/G
Water leaf	2.9×10^3	3.46
Spinach	2.8×10^4	4.51
Cabbage	1.6×10^3	3.20

Keys;-CFU/G-Colony forming unit per gram

Table2: Comparative distribution of biochemically and phenotypically confirmed *E. coli* isolated from the water leaf samples.

Source of samples	**No. of sample**	**No. with *E coli* (%)**	**No. without *E. coli* (%)**
CHOBA	52(40%)	3(60%)	
RUMUOSI	5	3(60%)	2(40%)
Total	10	5(50%)	5(50%)

Table 3: A comparative distribution of biochemically and phenotypically confirmed *E. coli* isolated from the spinach samples.

Source of samples	**No. of sample**	**No. with *E coli* (%)**	**No. without *E. coli* (%)**
CHOBA	5	4(80%)	1(20%)

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RUMUOSI	5	3(60%)	2(40%)
Total	10	7(70%)	3(30%)

Table 4: A comparative distribution of biochemically and phenotypically confirmed *E. coli* isolated from the cabbage samples

Source of samples	**No. of sample**	**No. with *E coli***	**No. without *E. coli***
		(%)	**(%)**
CHOBA	5	2(40%)	3(60%)
RUMUOSI	5	0(0%)	5(100%)
Total	10	2(20%)	8(80%)

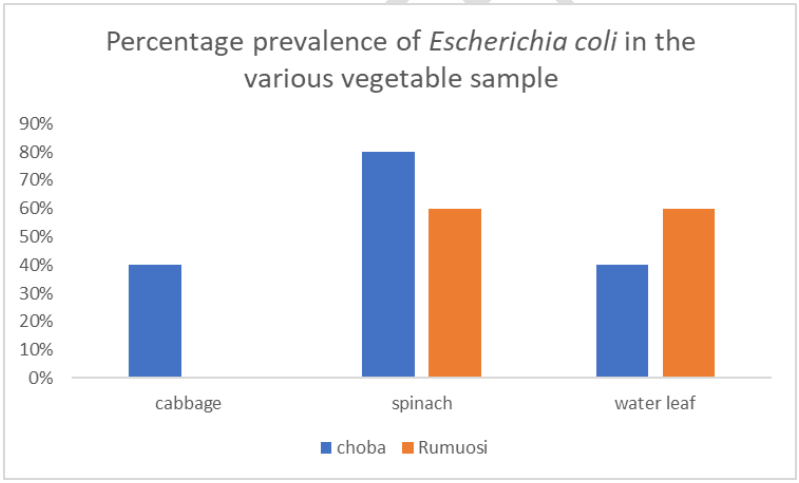

Fig.1: Percentage prevalence of *E coli*in the various vegetable samples obtained from Choba and Rumuosi markets

Table 5: Antibiotics susceptibility pattern of *E.coli* isolated from the various vegetable samples n=14

Comment [WPS_1657448]: This table did not indicate result for the various vegetables, instead it show the overall sensitivity pattern of the isolates to the antibiotics used.

	Resistant		Sensitive	
Antibiotics	% of Positive	inhibition zone (mm)	% of positive	inhibition zone (mm)
Tetracycline (30µg)	67.0	<14	33.0	>18
Ciprofloxacin (10µg)	16.2	<24	83.8	>28
Ampicillin (10µg)	72.3	<14	27.7	>16
Contrimozazole (30µg)	32.8	<15	67.1	>16
Chloramphenicol (30µg)	33.3	<12	66.7	>18
Amikacin (30µg)	49.5	<14	50.4	>17
Ceftriazone (10µg)	88.3	<15	11.6	>20
Gentamicin (30µg)	16.0	<12	84.0	>15
Streptomycin (10µg)	100.0	< 11	0.0	>15

Nalidixicacid(30µg)	42.9	<13	57.1	>16

Table 6: Antibiotics susceptibility pattern of strains of *E.coli* isolated from Spinach samples n=7

Comment [WPS_1657449]: This result should show number and percentage of resistant to each antibiotic used. Example, 2(45%).

	Resistant		Sensitive	
Antibiotics	% of Positive	inhibition zone (mm)	% of positive	inhibition zone (mm)
Tetracycline (30µg)	71.4	<14	28.6	>18
Ciprofloxacin (10µg)	28.6	<24	71.4	>28
Ampicillin (10µg)	57.1	<14	42.9	>16
Contrimozazole (30µg)	28.6	<15	71.4	>16
Chloramphenicol (30µg)	0 .0	<12	100.0	>18
Amikacin (30µg)	28.6	<14	71.4	>17
Ceftriazone (10µg)	85.7	<15	14.28	>20
Gentamicin (30µg)	28.6	<12	71.4	>15
Streptomycin (10µg)	100.0	< 11	0.0	>15
Nalidixicacid(30µg)	44.3	<13	55.7	>16

PLATE 1: Shows different zone diameter of inhibition of different Antibiotics
The right side of the plate shows different diameter of inhibition

Table 7: Antibiotics susceptibility pattern of *E.coli* isolated from the cabbage samples. n=2

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Antibiotics	Resistant		Sensitive	
	% of Positive	inhibition zone (mm)	% of positive	inhibition zone (mm)
Tetracycline (30µg)	**50.0**	**<14**	**50.0**	**>18**
Ciprofloxacin (10µg)	**0 .0**	**<24**	**100**	**>28**
Ampicillin (10µg)	**100.0**	**<14**	**0.0**	**>16**
Contrimozazole (30µg)	**50.0**	**<15**	**50.0**	**>16**
Chloramphenicol (30µg)	**100 .0**	**<12**	**50.0**	**>18**
Amikacin (30µg)	**50 .0**	**<14**	**0.0**	**>17**
Ceftriazone (10µg)	**100.0**	**<15**	**0.0**	**>20**
Gentamicin (30µg)	**0.0**	**<12**	**100.0**	**>15**
Streptomycin (10µg)	**100.0**	**< 11**	**50.0**	**>15**
Nalidixicacid(30µg)	**50.0**	**<13**	**50.0**	**>16**

Table 8: Antibiotics susceptibility pattern of strains of *E. coli* isolated from water leafsamples n=5

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Antibiotics	Resistant % of Positive	Resistant inhibition zone (mm)	Sensitive % of positive	Sensitive inhibition zone (mm)
Tetracycline (30µg)	80.0	<14	20.0	>18
Ciprofloxacin (10µg)	20.0	<24	80.0	>28
Ampicillin (10µg)	60.0	<14	40.0	>16
Contrimozazole (30µg)	20.0	<15	80.0	>16
Chloramphenicol (30µg)	0 .0	<12	100.0	>18
Amikacin (30µg)	20.0	<14	80.0	>17
Ceftriazone (10µg)	80.0	<15	20.0	>20
Gentamicin (30µg)	20.0	<12	80.0	>15
Streptomycin (10µg)	100.0	< 11	0.0	>15
Nalidixicacid(30µg)	40.0	<13	60.0	>16

Extended beta lactamase production pattern of *E.coli*isolated from the various sample sources

Table 9: Numbers of *E.coli*isolates positive for ESBLs production

Sources of isolates	No with Extended Spectrum βlactam
CHOBA	
Spinach	3 (75%)
Cabbage	1(50%)
Water leaf	1(50%)
RUMUOSI	
Spinach	1(33.3%)
Cabbage	0(0%)
Water leaf	1(33.3%)

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PLATE 2: Shows a negative result obtained by the disk diffusion test, absent of enhance zone of inhibition between one of the beta lactam disks and disk contains clavulanic acid (the center disk).

PLATE 3: Shows a positive result obtained by the 2-disk test, presence of enhance zone of inhibition between one of the beta lactam disks and disk contains clavulanic acid

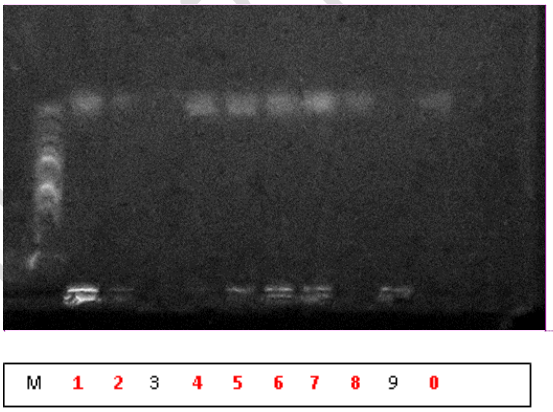

Plate 4: Gel picture of EAST virulence gene for *E. coli* showing positive and negative isolates. M is 100bp DNA ladder. Isolates highlighted red are positive for EAST virulence gene while black are negative

Comment [WPS_1657454]: Should be properly label, indicating the genes of interest and it molecular weight, ladder and the negative isolates respectively

DISCUSSION

The result ofgreen metallic sheen colony on eosin methylene blue agar obtained from the three leaf samples is represented on table1.Water leaf samples had a mean count of 2.9×10^3,spinach sample mean count was2.8×10^4 Cfu/g AND the highest, while the lowest meancount was obtained from cabbage samples;1.6×10^3Cfu/g.ThePercentage prevalence of *E coli* in the various vegetable samples obtained from Choba and Rumuosi markets are presentedon figure 1. Water leaf and cabbage samples obtained from Choba market had the same prevalence of 40%, while none was isolated from cabbage samples bought from Rumuosi market. Water leaf obtained from Rumuosi had 60% prevalence while Spinach samples obtained from Chobahad prevalence of 80% which was the highest obtained from the two markets and the three leafy greens.Spinach samples was found to be most contaminated of all the three leafy greensamples with a mean count of 2.8×10^4cfu/g (table 1)and highest prevalence rate of 80%(table .4) while Cabbage sampleswas contaminated with a count of 1.6×10^3cfu/g (table 1) and 40% prevalence (table 4) which is the lowest. This might be due to the facts that the vegetable creeps on the soil. According to Sayah*et al.*, 2005,*E.coli*have been isolated on several occasions from human and animal feaces and also FROM the carcass of cattle, sheep, horses, pigs, turkeys, dogs and a variety of wild animals which are dumped or used as manure on the soil.This result is consistence with the work of (Atnafie*et al.*, 2017) whose study revealed that several strains of *E.coli* can be isolated from the surface of raw salad vegetables which includes spinach and cabbage. The low prevalence rate of *E.coli*in cabbage samples2(20%) out of 10 samples analyzed (table 4) agreed with (Seow*et al.*, 2012) who reported that *E.coli* was found cabbage and prevalence was less.The high mean count may indicate the poor handling practice during storage and point of selling. Several studies reported that the major source of microbial contamination was an anthropogenic disturbance in human, animals and irrigation water (Oliveira *et al.* 2011).

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Table .5 shows the antibioticresistance and susceptibility patterns of 14 isolates of *E. coli* isolated from the various sample sources. Resistance was highest for ampicillin (100%) tetracycline had (62.5%) and sensitivity was highest with ciprofloxacin (81.25%), and gentamicin (68.7%), Comparison of the antibiotic resistant pattern of isolates from each sample type was shown in table 6-9. All the *E coli* isolated from spinach samples, exhibited 100% resistance to ampicillin. Isolates from cabbage also shows100% resistance to ampicilin. Multi drug resistance was common amongst the isolates. Fifty percent of the all the isolates were resistant to fiveantibiotics.The *E.coli*isolated from this study displayed resistance to one or more antimicrobial agents including streptomycin, tetracycline, ampicillin, amikacin, andchloramphenicol.Resistance was highest for streptomycin (100%) followed by ceftriaxone (88.5%) while susceptibility was highest for gentamycin (84%) and lowest for ceftriaxone (Table 4-8). The development of resistance to antimicrobial is known to occur through stable genetic change heritable from generation to generation through specific mechanisms including mutation, transduction, transformation and or conjugation (Cohen, 2000).Sensitive and resistance patterns of the 14 identified*E.coli* isolated from different vegetable samples tested against the 10 antibiotics were shown on Table 4-.8.. The bacterial isolates showed considerable level of resistant against streptomycin (100%) tetracycline had average of (62.5%) and ceftriazone had (88.5%). Susceptibility was highest withgentamicin (84%) and ciprofloxacin (83%). This agrees with the studies carried out by (Musa *et al*., 2010) who reported that all the isolates of *E.coli* from cow were susceptible to ciprofloxacin.(Aibinu*et al*., 2007) also reported that 12(66.6%) out of 18 isolates from animal and human sources are susceptible to ciprofloxacin. Comparison of the antibiotic resistant pattern of isolates from each sample type was shown in table 4-8. Out of the 2 isolated from cabbage samples, 2(100%) showed resistant to tetracycline while all had 100% susceptibility to ciprofloxacin, Strains from univalve showed 100% resistant to ampicilin. Multi drug resistance was

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common amongst the isolates. Fifty percent of all theisolates were resistant to five antibiotics and also fifty percent were resistant to more than two antibiotics.

Table 9 revealsthe extended beta lactamase pattern of *E. coli* isolated from the various sample sources. Spinach samples obtained from Choba market had the highest percentage of ESBL positive *Escherichia coli* (75%)while cabbage and water leaf had the same percentage of ESBL positive *E coli*(50%). *E. coli* isolated from spinach and waterleaf samples obtained from Rumuosi had the same percentage of ESBL producing *Escherichia coli* (33.3%) samples, A number of studies have assessed the occurrence of ESBLs among the family of Enterobacteriaeceae (Singhal*et al*. 2004;Rahal*et al*.,2002). In this study 7(50%) out 14*E.coli*, produced ESBLs. This could be as a result of mutationswithin the structural genes encoding the older enzymes, giving rise to derivatives that possessed an extended substrate profile compared with that of the parentalenzymes (Cohen,2000).The Gel picture of EAST (enterohaemorrhagic and enteropathogenic) Virulence gene for *E. coli*.(Plate 4)shows that out of the ten isolates subjected to the test, eight were positive for Enteroheamorrhagic. Paddock *et al.*,(2013) reported that *E. coli* isolated from vegetables, cattle and human sources were positive for ast,A gene which is frequently associated with diarrhea. Seven (50%) of the total isolates produced Extended β-lactamase. A number of studies have assessed the occurrences of ESBLs among members of the family Enterobacteriaeeae, (Singhalet al.2004,Araque, 2013 andBezabih et al.2020). About 31.7% *E.coli* isolated from vegetable farms in Imo State, Nigeria produced ESBLS (Akujobiet al. 2008). A large number of outbreaks of infection due to ESBL-producing organisms have been described on every continent of the globe (Mark *et al.*,2003 and Tankoet al. 2020). Clinical outcomes data indicate that ESBLs are clinically significant and, when detected, indicate the need for the use of appropriate antibacterial agents (Akulobiet al.2010).

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The Gel picture of EAST (Enterohaemorrhagic and Enteropathogenic) virulence gene for *E. coli*.is shown on Plate 4. Out of the ten isolates subjected to the test, eight were positive for Enteroheamorrhagic and Enteropathogenicvirulence gene while the remaining two were negative.Ateba&moses, (2010) also detected *Escherichia coli* 0157:H7 virulence genes in isolates forms beef, pork, water, human and animal species in Northwest province of South Africa

Conclusion

The study has established the presence of pathogenic *E.coli* (Enterohaemorrhagic) which are Shiga toxin producersTHAT AREHighly virulent with low infections doses.Spinach samples was found to be most contaminated of all the three leafy greensamples with a mean count of 2.8×10^{4}cfu/g and highest prevalence rate of 80% while Cabbage sampleswas contaminated with a count of 1.6×10^{3}cfu/g (table .1) and 40% prevalence which is the lowest.

Out of the ten isolates subjected to detection of virulence gene, eight isolates were positive for the virulence genes namely Enteropathogenic (EPEC) and Enterohemorrhagic (EHEC) genes which makes this organism a dangerous pathogen

The presence of this pathogen indicates that consumers of these vegetables in some parts of Rivers State are at risk of acquiring this pathogen which could be transferred from person to person, and animal to animals.

The poor sanitary condition of practices observed in the various markets could be responsible for spread of the pathogen.

Recommendation

When patients present with food poisoning symptoms the causative agents should be properly identified and sources of food be identified for epidemiological information and data base for referencing.

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Consumers shouldavoid indiscriminate eating of foods sold in open places and cook their food properly.

The indiscriminate use of antibiotics should also be discouraged since it is implicated as one of the reasons for multiple resistance to antibiotics by pathogens.

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