Antibiotic Susceptibility Pattern of Sorbitol and Non-Sorbitol Fermenting *E. coli* Isolated from Vegetable Sources in Selected Markets at Keffi, Nasarawa State, Nigeria

****Nggada, N.J *Gberikon, G.M., *Ogbonna, I.O**
*Department of Microbiology, College of Science,University of Agriculture Makurdi, Benue state
**Department of Microbiology, Faculty of Natural and Applied Sciences, Nasarawa State University, Keffi
Corresponding Author: gracegberikon@yahoo.com

Abstract
Antibiotic susceptibility pattern of sorbitol (SFE) and non-Sorbitol (NSFE) fermenting *E. coli* isolated from vegetable sources in selected marketsKeffi, Nasarawa State, Nigeria was ascertained. Twenty (20) samples each from pumpkin leaves (*Telfariaoccidentalis*), lettuce (*Lactuca sativa*), spinach (*Amaranthushybridus)*, cabbage (*Brassica oleracea*) and carrot (*Daucuscarota*) making a total of hundred (100) samples were packaged separately in sterile polythene bags and immediately transported to Nasarawa state University Microbiology Laboratory for analyses. Sorbitol and non-sorbitol fermenting *E.coli* were isolated and identified from the samples using some standard microbiological and biochemical methods. Antibiotic susceptibility test using disc diffusion methods as described by Clinical and Laboratory Standards Institute (CLSI) and the confirmatory test for extended spectrum β-lactamase (ESBL) production in NSFE and SFE isolates jointly resistance to both cefotaxime and ceftazidime from vegetables were carried out using double disc synergy method. Results showed that NSFE isolates were more susceptible to gentamicin (100.0), ciprofloxacin (88.2%) and imipenem (76.5%) whereas the SFE isolates were more susceptible to gentamicin (92.7%), ciprofloxacin (80.5%) and imipenem (73.2%). The detection of ESBL producing NSFE and SFE isolates jointly resistant to both cefotaxime and ceftazidime was 100.0%.Multiple antibiotic resistance(MAR) index showed that there was statistically significant ($p<0.05$) differencein the antibiotic susceptibility pattern as observed in this study.Isolatesshowed multiple antibiotic resistance with MAR index of > 0.2 across the entire antibiotics.Indiscriminate use of antibiotics in human and animal feeds should be prohibited, so that resistance to antibiotics will be reduced to a minimal level.

Keywords: Antibiotics, Susceptibility, Vegetables, *E.coli*, Cefotaxime, Ceftazidime

1. INTRODUCTION

*Escherichia coli*constitute normal flora of the human intestine, some can be grouped into sorbitol fermenting (SF) and non-sorbitol fermenting (NSF) (Ngwai*et al*., 2014). *Escherichia coli* O157: H7 is suspected to be non-sorbitol (Ngwai*et al*., 2014) that causes diarrhea and hemolytic uremic syndrome in highly vulnerable groups (Annet and Ole, 2009). Other infection that have been implicated by*E. coli*apart from diarrhea are sepsis, meningitis and urinary tract infections especially in immune compromised people (Croxen*et al.,* 2010).There are six pathogenic strains of *E. coli* reported to cause diarrhea, and they are implicated in food poisoning (Annet and Ole, 2009). Indeveloping countries diarrhea caused by *E. coli* is one of the major public health concernand this has contributed exceedingly to high mortality rate (Jouini*et al*., 2007).Antimicrobial resistance have been reported in cases arising from infections (Ngwai*et al*., 2014).

Resistance to β-lactam antibiotics has become a public health issue, especially from organisms originating from foods and animal feeds (Jouini*et al*., 2007), leading to point-break situation where no antibiotic treatment option, remains. These situations are of serious concern in developing countries where enteropathogens are frequently encountered and cause life threatening infection especially among children.

2. MATERIALS AND METHODS

Sample collection

Twenty (20) samples each of vegetables such as cabbage (*Brassica oleracea*), spinach (*Amaranthushybridus*), lettuce (*Lactuca sativa*), pumpkin leaves (*Telfairiaoccidentalis*)and Carrots (*Daucuscarota*) making a total of hundred (100) were randomly purchased directly from vegetable sellers in Keffi main market, NasarawaState, Nigeria. These vegetable samples were collected using sterile polythene bags and were transported to Microbiology Laboratory, Nasarawa State University Keffi, Nigeria in an ice pack for analysis.

Preparation of vegetable samples

Vegetable samples were prepared by modification of the method described by Ngwai*et al.* (2014). Samples were sliced using a sterile knife and exactly 10.0 g of each sliced vegetable

samples were dispensed into 90 ml of sterile distilled water in 250 ml sterile conical flask, it was vigorously shaken and allowed to stand for 4 hours before inoculation.

Preparation of Media and Inoculation for Isolation of *Escherichia coli*

Preparation of Culture media:

Nutrient Agar, Mueller-Hinton Agar, Simmons Citrate Agar, Peptone water, Eosin Methylene Blue (EMB), MacConkey agar, Tryptone soy broth, Sorbitol-MacConkey agar were all prepared according to manufacturer's standards.

Isolation of Sorbitol and Non-Sorbiol Fermenting *Escherichia coli*

Exactly, 1ml of each prepared vegetable samples such as cabbage, spinach, lettuce, pumpkin leaves and carrots were inoculated into 9 ml of Tryptone Soy Broth (TSB) and incubated at 37 ^{o}C for 24h. A loopful of the inoculum in TSB was streaked on Sorbitol MacConkey agar and were incubated for 24 h at 37^{o}C. Colourless (non-sorbitol fermenting) and pinkish (sorbitol fermenting) colonies were sub-cultured on EMB agar plates and incubated at 37 ^{o}C for 24 h. Greenish metallic sheen colonies that were grown on EMB agar plates after 24 h incubation were selected as suspected *E. coli* (Ngwai*et al*., 2014)

Identification of *Escherichia coli*

Standard biochemical tests such as Gram-staining, Indole Test, Methyl Red Test,Voges-Proskauer Test, Citrate Test were carried out adopting methods of Cheesebrough (2006)

Antibiotic Susceptibility Test

The antibiotic susceptibility testing was carried out using Kirby-Bauer disc diffusion method modified by CLSI (2014). Four (4) variants colonies of SF and NSF *E.coli* isolates were inoculated into 5 ml of sterile normal saline in a test-tube, and the turbidity of the bacteria suspension was adjusted equivalents to turbidity of 0.5 McFarland's standard. The McFarland's standard was prepared as follows: 0.5 ml of 1.172 % (w/v) $BaCl_2 ._2H_2O$ was added to 99.5ml of 1% (v/v) H_2SO_4. A sterile cotton swab stick was soaked in the adjusted SF and NSF *E.coli* suspension and was streaked on Mueller Hinton agar (MHA) plates. The antibiotic discs were

placed aseptically on Mueller-Hinton agar plates containing the *E.coli* isolates. The plates were allowed to stand for 1 h for pre-diffusion at room temperature (25°C) before they were incubated at 37^{o}C for 24 h. The diameter zone of inhibition (in mm) was determined; using meter rule and the result was interpreted in accordance with CLSI (2014). Antibiotic disks from Oxoid Ltd (England) that were used include: gentamicin (10µg), cefuroxime (30 µg), Amoxicillin-Clavulanate (30µg), Perfloxacin (10µg), Streptomycin (30 µg), Ampicillin (30µg), Ciprofloxacin (5µg), Chloramphenicol (30µg) and Sulphonamide/sulphomethoxazole (30 µg), Ceftazidime (30 µg), Cefotaxime (30 µg) and Cefoxitin (30 µg).

Confirmatory Test for Extended Spectrum β-Lactamase Production

The confirmatory test for ESBL production by the isolates jointly resistant to both cefotaxime and ceftazidime antibiotics were carried out on SF and NSF *E. coli* isolates whose diameter zone of inhibition were ≥24mm and ≥22mm using Double Antibiotic Synergy Test (DAST) Jafari*et al.*(2009). Swab stick was soaked in a standardized suspension (10^{5}Cfu/ml) of SF and NSF *E. coli* jointly resistance to both cefotaxime and ceftazidime antibiotics and streaked on MHA plates and 30µg of Amoxicillin-Clavulanate disks were placed at the centre; and 30µg ceftazidime and 30µg cefotaxime disks 20 mm away from the Amoxicillin-Clavulanate disks. The plates were allowed to stand for 1 h for pre-diffusion at room temperature before they were incubated at 37^{o}C for 24 h. Isolates with increase in zone of inhibition of both ceftazidime and cefotaxime towards Amoxicillin-Clavulanate was confirmed as ESBL producers.

Statistical Analysis

The data that was obtained in this study on frequency of occurrence of sorbitol fermenting and non-sorbitol fermenting *Escherichia coli* isolates were subjected to Chi-square test using Statistical Package for Social Sciences (SPSS) version 21.0 and the significance was determined at 5% probability.

Table 1: Antibiotic Susceptibility Pattern of Sorbitol and Non-Sorbitol Fermenting *Escherichia coli* Isolated from Selected Vegetables Sold in Keffi Market, Nasarawa State, Nigeria

Antibiotics	Disks content (µg)	No. (%) Susceptibility	
		NSF *E. coli* (n=17)	SF *E. coli* (n=41)
Ampicillin (AMP)	30	0(0)	0(0)
Amoxycillin/clavulanic acid (AMC)	30	0(0)	0(0)
Ceftazidime (CAZ)	30	5(29.4)	19(46.3)
Cefotaxime (CTX)	30	2(11.8)	12(29.3)
Cefoxitin (FOX)	30	2(11.8)	4(9.8)
Gentamicin (CN)	10	17(100.0)	38(92.7)
Ciprofloxacin (CIP)	5	15(88.2)	33(80.5)
Sulphamethoxazole/ Trimethoprim (SXT)	25	4(23.5)	12(29.3)
Streptomycin (S)	30	1(5.9)	1(2.4)
Imipenems (IPM)	30	13(76.5)	30(73.2)
		Fvalue= 4.2121	pvalue = 0.0550

KEY: SFE=Sorbitol Fermenting *Escherichia coli*, NSFE=Non Sorbitol Fermenting *Escherichia coli*

Table 2:Multiple Antibiotic Resistance (MAR) Index of Sorbitol Fermentingand Non- Sorbitol Fermenting *Escherichia coli* Isolates from Vegetables Sold in Keffi Main Market, Nigeria

No. of antibiotics Resistance to (a)	No. of antibiotics tested (b)	MAR Index	No. (%) MAR Isolates	
			SFE(n=41)	NSFE(n=17)
9	10	0.9	2(4.9)	0(0)
8	10	0.8	8(19.5)	2(11.8)
7	10	0.7	12(29.3)	6(35.3)
6	10	0.6	8(19.5)	7(41.2)
5	10	0.5	9(22.0)	2(11.8)
4	10	1.4	2(4.9)	0(0)

Fvalue = 1.2482 pvalue = 0.2900

Table 3: General Antibiotic Susceptibility Pattern in Sorbitol and Non Sorbitol Fermenting *E. coli* from Vegetable Samples

S/N	ISOLATE NO.	CN	CIP	CTX	FOX	CAZ	AMP	AMC	SXT	S	IPM
1	CB4	21mm	0mm	0mm	0mm	15mm	0mm	0mm	0mm	11mm	27mm

2	CB1	28mm	28mm	10mm	0mm	0mm	0mm	0mm	20mm	0mm	25mm
3	LT2	24mm	0mm	0mm	0mm	20mm	0mm	0mm	0mm	10mm	28mm
4	C4	20mm	30mm	0mm	0mm	18mm	0mm	0mm	0mm	0mm	26mm
5	CB2	23mm	29mm	0mm	0mm	20mm	0mm	0mm	20mm	0mm	22mm
6	CB3	30mm	22mm	20mm	0mm	28mm	0mm	0mm	23mm	13mm	25mm
7	SP6	25mm	20mm	0mm	0mm	20mm	0mm	0mm	0mm	0mm	30mm
8	C1	19mm	30mm	30mm	0mm	22mm	0mm	0mm	20mm	0mm	24mm
9	CB11	28mm	32mm	20mm	0mm	24mm	0mm	0mm	25mm	10mm	26mm
10	LT8	20mm	30mm	0mm	0mm	15mm	0mm	0mm	0mm	0mm	25mm
11	CB6	28mm	38mm	0mm	0mm	20mm	0mm	0mm	0mm	0mm	32mm
12	LT1	22mm	38mm	0mm	0mm	22mm	0mm	0mm	0mm	7mm	27mm
13	C6	24mm	32mm	16mm	0mm	22mm	0mm	0mm	0mm	0mm	26mm
14	C2	0mm	34mm	0mm	0mm	23mm	0mm	0mm	12mm	0mm	22mm
15	C8	30mm	30mm	20mm	0mm	26mm	0mm	0mm	0mm	0mm	28mm
16	PK2	25mm	30mm	0mm	0mm	24mm	0mm	0mm	0mm	0mm	30mm
17	SP10	29mm	32mm	13mm	16mm	28mm	0mm	0mm	19mm	0mm	28mm
18	SP11	24mm	38mm	23mm	0mm	17mm	7mm	0mm	0mm	0mm	33mm
19	C5	27mm	34mm	23mm	20mm	0mm	0mm	9mm	26mm	0mm	26mm
20	C10	24mm	36mm	18mm	0mm	23mm	0mm	8mm	0mm	0mm	32mm
21	LT5	24mm	30mm	15mm	0mm	0mm	0mm	0mm	0mm	0mm	29mm
22	C18	28mm	26mm	13mm	0mm	25mm	0mm	0mm	0mm	0mm	26mm
23	CB10	27mm	38mm	24mm	0mm	0mm	0mm	0mm	10mm	0mm	32mm
24	LT6	21mm	35mm	0mm	0mm	24mm	0mm	0mm	0mm	0mm	30mm
25	CB5	25mm	38mm	23mm	0mm	18mm	0mm	0mm	0mm	0mm	28mm
26	LT7	38mm	24mm	24mm	15mm	24mm	0mm	0mm	18mm	13mm	25mm
27	CB9	27mm	29mm	29mm	0mm	8mm	0mm	0mm	0mm	0mm	26mm
28	SP4	28mm	40mm	28mm	0mm	14mm	0mm	0mm	0mm	0mm	34mm
29	SP1	12mm	10mm	0mm	0mm	12mm	0mm	0mm	0mm	10mm	28mm
30	LT3	27mm	32mm	0mm	0mm	25mm	0mm	0mm	28mm	0mm	30mm
31	C9	18mm	30mm	0mm	0mm	11mm	0mm	0mm	0mm	0mm	24mm
32	SP3	28mm	26mm	18mm	12mm	0mm	0mm	0mm	0mm	0mm	24mm

KEY: CN=Gentamycin, CTX=Cefotaxime, FOX=Cefotaxitin, CIP=Ciprofloxacin, CAZ=Ceftazidime, AMP=Ampicillin, AMC=Amoxycillin/Clavulanic Acid, SXT=Sulphamethoxozole/Trimethoprim, S=Streptomycin, IPM=Imipenems, CB=Cabbage, LT=Lettuce, SP=Spinach, PK=Pumpkin, C=Carrot

Table 3 cont'd: General Antibiotic Susceptibility Pattern for Sorbitol and Non Sorbitol Fermenting *E. coli* from Vegetable Samples

S/N	ISOLATE NO.	CN	CIP	CTX	FOX	CAZ	AMP	AMC	SXT	S	IPM
33	LT11	29mm	25mm	22mm	0mm	0mm	0mm	0mm	21mm	0mm	30mm
34	LT4	25mm	30mm	0mm	0mm	19mm	0mm	0mm	0mm	10mm	26mm
35	PK7	29mm	18mm	0mm	0mm	18mm	0mm	0mm	0mm	0mm	28mm
36	PK9	20mm	34mm	0mm	0mm	17mm	0mm	0mm	0mm	0mm	25mm

37	CB12	19mm	30mm	14mm	0mm	18mm	0mm	0mm	0mm	0mm	26mm
38	LT12	25mm	24mm	0mm	0mm	0mm	0mm	0mm	0mm	0mm	27mm
39	LT10	20mm	24mm	0mm	0mm	22mm	0mm	0mm	0mm	0mm	25mm
40	C11	23mm	25mm	20mm	28mm	21mm	0mm	0mm	18mm	10mm	22mm
41	SP9	25mm	25mm	21mm	0mm	22mm	0mm	0mm	23mm	27mm	29mm
42	PK1	28mm	30mm	26mm	20mm	25mm	0mm	0mm	0mm	0mm	25mm
43	LT9	15mm	19mm	0mm	0mm	10mm	0mm	0mm	0mm	0mm	27mm
44	PK3	28mm	30mm	23mm	0mm	0mm	0mm	0mm	18mm	0mm	28mm
45	PK4	19mm	29mm	14mm	0mm	0mm	0mm	0mm	0mm	0mm	28mm
46	PK6	21mm	28mm	0mm	0mm	19mm	0mm	0mm	0mm	0mm	29mm
47	CB8	25mm	18mm	25mm	0mm	22mm	0mm	10m	20mm	0mm	32mm
48	SP8	24mm	25mm	16mm	22mm	21mm	0mm	0mm	21mm	0mm	22mm
49	CB13	26mm	28mm	10mm	19mm	24mm	0mm	0mm	0mm	0mm	28mm
50	PK10	21mm	24mm	20mm	0mm	25mm	0mm	0mm	0mm	0mm	27mm
51	C3	23mm	0mm	0mm	0mm	22mm	0mm	0mm	0mm	0mm	31mm
52	SP5	22mm	32mm	23mm	0mm	18mm	0mm	0mm	0mm	0mm	30mm
53	PK8	23mm	24mm	0mm	13mm	23mm	0mm	0mm	0mm	0mm	28mm
54	C7	24mm	23mm	12mm	0mm	23mm	0mm	0mm	0mm	0mm	26mm
55	C12	25mm	26mm	0mm	0mm	23mm	0mm	0mm	25mm	15mm	28mm
56	PK5	23mm	25mm	0mm	0mm	21mm	0mm	0mm	0mm	0mm	28mm
57	SP7	23mm	20mm	23mm	20mm	20mm	0mm	0mm	0mm	11mm	28mm
58	C19	14mm	20mm	23mm	17mm	0mm	0mm	0mm	0mm	10mm	25mm
		≥15mm	≥21mm	≥23mm	≥18mm	≥22mm	≥17mm	≥18	≥16	≥15	≥26
		S=25+30 55	S=21+27 48	S=4+4 8	S=5+1 6	S=9+9 18			S=8+8 16	S=2	S=19+24 43

KEY: CN=Gentamycin, CTX=Cefotaxime, FOX=Cefoxitin, CIP=Ciprofloxacin, CAZ=Ceftazidime, AMP=Ampicillin, AMC=Amoxycillin /Clavulanic Acid, SXT=Sulphamethoxozole /Trimethoprim, S=Streptomycin, IMP=Imipenems, CB=Cabbage, SP=Spinach, LT=Lettuce, PK=Pumpkin, C=Carrot, S= Susceptibility

3. DISCUSSION

Consumption of vegetables has increased over the years (CDC, 2013) because of its high nutritive value. Though, the occurrence of sorbitolfermenting *E.coli*(SFE) and non-sorbitol (NSFE) fermenting *E.coli* from vegetables recorded in this study was higher than the study reported by Ngwai*et al*. (2014) and Solomon *et al.* (2002). The occurrence of SFE and NSFE may be due to the fact that most vegetables in Nigeria are grown with water that are contaminated with this organism from feacal matter of humans andanimal origin (Solomon *et*

al., 2002) and this agrees with the earlier study by (Ngwai*et al*., 2014).The high susceptibility of NSFE and SFE to ciprofloxacin, gentamicin and imipenem as observed in this study was expected and this also justifies their use as broad spectrum antibiotics for treatment of Gram negative bacterial infection. Ciprofloxacin and imipenems are very expensive, gentamicin is in an injectable form and because of the cost of ciprofloxacin and Imipenems and the discomfort of gentamicin injection when administered parentally, it is likely that such antibiotics may not have been abused (Ngwai*et al*., 2014). It was observed in this study that NSFE and SFE were less susceptible to the third generation Cephalosporin (Cefotaxine and Ceftazidime) and this however seems to disagree with the study earlier reported by Nkene*et al*. (2016). The high susceptibility pattern of NSFE and SFE to Imipenems justifies the use of Imipenems as the last choice of β-lactam antibiotics for treatment of Gram negative bacterial infection. The production of ESBL by NSFE and SFE jointly resistant to both Cefotaxine and Ceftazidime observed in this study was not out of place, this is in tandem with the study reported by Jacoby *et al.* (2009) and Nkene*et al.* (2016). The high detection of ESBL producing *E. coli* in vegetable samples observed in this study is in agreement with the study earlier reported by Anette and Ole *(*2009). The MAR index were statistically significant as observed in this study, it shows that the isolates are multiple antibiotics resistant with MAR index of > 0.2 across the entire antibiotics test which is in agreement with the study reported by Nkene*et al.* (2016).

4. Conclusion

It was concluded from this study that SFE and NSFE were high in vegetables analyzed from this study. It was established from this study that NSFE and SFE isolates from vegetables purchased from Keffimain market were more susceptible to Ciprofloxacin, Gentamicin and Imipenems and this however implies that the antibiotics mentioned above may be useful for treatment of *E. coli* infection. In addition, all the *E. coli* isolates jointly resistance to both Cefotaxine and Ceftazidime were ESBL producers. Therefore indiscriminate use of antibiotics in human and animal feeds should be discouraged in other to curb resistance.

REFERENCES

Anette MH and Ole E.H (2009). Human Health Hazards from AntimicrobialResistant*Escherichia coli* of Animal Origin. *Clinical Infectious Diseases*, 48:916–21.

CDC, (2013). On microbial resistance Geneva.

Cheesbrough, M. (2006). *District Laboratory Practice in Tropical Countries,* Part II. Cambridge University press, Cambridge, U.K.pp442.

CLSI (2014). Performance Standards for Antimicrobial Susceptibility Testing: 24th Informational Supplement M100-S24. Wagne, PA: Clinical and Laboratory Standards Institute.

Croxen, M. A., and Finlay, B. B. (2010). Molecular mechanisms of *Escherichia coli* pathogenicity. *Natural Revised Microbiology;* 8:26-38.

Jacoby, G. A. and Medeiros, A. A. (1991). More extended- spectrum β-Lactamase.*Antimicrobial Agents and Chemotherapy*, 35: 1697-1704.

Jafari, F. Hamidian, M., Rezadehbakhshi, M., Doyle, M., Salmanzadeh-Ahrabi, S. and Derakhshan, F. (2009). Prevalence and antimicrobial resistance of diarrheagenic*Escherichia coli* and *Shigella*species associated with acute diarrhea in Tehran, Iran. *Canadian Journal of Infectious Diseases in Medical Microbiology;* 20: 56-62.

Jouini, A., Vinue, L and Slama, K. B, (2007). Characterization of CTX-M and SHV extended-spectrum β-Lactamases and Associated Resistance genes in *Escherichia coli* strains of food samples in Tunisia. *Antimicrobial Agents and Chemotherapy*, 60:1137–41.

Lesley-Ann, C Uchechukwu. U. Nwodo, AI. Okoh, RN. and Ezekiel G. (2014). Die durchohrung von membranendurchpilzfaden. Jahrbwissot 28.269289.

Ngwai, Y.B., Gyar, S.D., Pennap, G.R.I., Makut, M.D., Ishaleku, D., Corosi S.M., Nkene, I.H and Uzoaegwai, I. U. (2014). Antibiogram of non-sorbitol fermenting *Escherichia coli* from sources and stool in Keffi, Nigeria. *NSUK journal of Science and Technology*, vol. 4 (1 and 2): 152-163

Nkene, I. H., Ngawi, Y.B., Omede, M. U., Samuel, J., Envuladu, E. Y. and Abimiku, R. H. (2016). Extended spectrum β-lactamase producing Escherichia coli from urine of

symptomatic and asymptomatic subjects in Keffi, Nigeria. *International Journal ofResearch Studies in Biosciences*, 3(12): 1-5.

Solomon, E.B., Yaron S. and Mathew K. R. (2002). Transmission of *Escherichia coli* 0157:H7from a contaminated manure and irrigation water to Lettuce and plant and its subsequent internationalization. Applied and Environmental Microbiology. 68: 397-400.