ANTIBIOGRAM OF NON-SORBITOL FERMENTING *ESCHERICHIA COLI* ISOLATED FROM ENVIRONMENTAL SOURCES IN KEFFI, NIGERIA

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ABSTRACT

Non-sorbitol fermenting Escherichia coli, particularly O157:H7, are major public health concern. This study was undertaken to isolate and identify non-sorbitol fermenting (NSF) E. coli from stool samples and food sources in Keffi metropolis. Four hundred and thirty (430) samples of vegetables (100), raw beef (110), undercooked beef (60), local fresh milk product ("Nono": 50), cow dung (10) and human stool (100) were collected from five different locations in Keffi metropolis and NSF E. coli was isolated and confirmed by culture on Sorbitol MacConkey agar, Eosine Methylene Blue agar and biochemical tests namely, Indole, Methyl Red, Voges-Poskauer and citrate test (IMViC). Antibiotics susceptibility tests using standard procedure were carried on the isolates. E. coli was detected in 298 (69.3%) samples. Of the positive samples, 64 (21.5%) were NSF E coli with high detection in raw beef (43.8%) and cow dung (40.0%); and 234 (78.5%) were sorbitol-fermenting (SF) strains with high detection in all sources. The NSF E. coli were highly susceptible to septrin (79.7%), peflacine (68.8%), streptomycin (71.9%), gentamicin (62.5%) and ofloxacin (70.3%); but less to ampicillin (12.5%), augmentin (32.8%), ceporex (32.8%), nalidixic acid (43.8%) and ciprofloxacin (48.4%). There were 46 different resistance phenotypes with AU, CPX, SXT, S, PN, CEP, OFX, NA, PEF; AU, CPX, PN, CEP, NA; AU, PN, CEP, NA, CN; PN, CEP, NA, CN; and CPX, PN, NA dominating at 4.7 % each. MAR was observed in all the isolates with resistance to 4 and 5 antibiotics being more frequent at 23.4% and 21.9% respectively. The lowest MAR index was 0.3. In conclusion, non-sorbitol fermenting E. coli was shown to contaminate some cabbage vegetables, raw beef, undercooked beef, and "Nono" consumed in Keffi metropolis. The NSF E. coli were more susceptible to septrin, aminoglycosides and ofloxacin; and originate from an environment where antimicrobials are freely available and misused.

Keywords: *Antibiogram; Escherichia coli; Non-sorbitol fermenting*

INTRODUCTION

Escherichia coli is a gram-negative rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms. Most *E. coli* strains are harmless but some such as the serotype O157:H7 can cause serious food poisoning in humans and are occasionally responsible for product recalls (Moses *et al*., 2006; Karch *et al*., 2005). The harmless strains are part of the normal flora of the gut, and can benefit their host by producing Vitamin K_2 (Rendon*et al*., 2007) and by preventing the establishment of pathogenic bacteria within the intestine (Hudault *et al*., 2001). *Escherichia coli* colonizes the infant gastro intestinal tract within hours of life and thereafter, together with the host, derive mutual benefit (Moses and Martinko, 2006). *E. coli* and related bacteria constitute above 0.1 % of gut flora and faecal-oral transmission is a major route through which pathogenic strains of the bacterium causes diseases (Eckburg *et al*., 2005). Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections and neonatal meningitis; in rare cases, virulent strains are also responsible for haemolytic uremic syndrome, peritonitis, mastitis, septicaemia and gram-negative pneumonia (Thompson, 2007; Todar, 2007). Most (93%) strains of *E. coli* ferment sorbitol to produce acid; but some such as *E. coli* O157:H7 do not (Wells *et al*., 1983).

Transmission routes of food-borne pathogenic non-sorbitol fermenting *E. coli* include: vegetable, unpasteurized fruit drink, milk, untreated water and meat products (such as hamburger, 'suya' and salami), direct from animals to animal keepers (Morgan *et al*., 1988; Chapman *et al*., 1993; Keene *et al*., 1994; Milne *et al*., 1999; Galland *et al*., 2001; O'Connor, 2002; Todar, 2007; Onono *et al*., 2010).

In Nigeria, food-borne pathogenic non-sorbitol fermenting *E. coli* has been isolated in several places (Akinyemi *et al*., 1998; Coker *et al*., 2000; Olorunshola *et al*., 2000; Smith *et al*., 2003; Moses *et al*., 2006). No report from Keffi is available on the isolation of non-sorbitol fermenting *E. coli* from various food sources. This study seeks to isolate, identify and classify into sorbitol-fermenting and non-fermenting groups, *E. coli* from human stool, cow dung and food sources such as raw beef, and undercooked meat, vegetable and "Nono" (local fresh milk product) in Keffi, Nigeria. In addition, the study will test the susceptibility of the isolates to commonly used antimicrobial agents.

MATERIALS AND METHODS

Study Area and Sample Collection

The study area is Keffi metropolis, an ancient town located in Nasarawa West Senatorial Zone of the North Central State of Nasarawa State in Nigeria. Samples were collected from five different locations in Keffi metropolis namely: Agwan Lambu, Keffi market, Angwan Mada, G.R.A, and Agwan Tofa. A total of 430 samples consisting of cabbage vegetable (100), raw beef (110), undercooked beef (60), "Nono" (50), cow dung (10) and human stool (100) were collected in all using sterile samples bottles. The cow dung samples were collected from Keffi Abattoir; while the human stool samples were from patients attending Federal Medical Centre Keffi.

Media and Antimicrobial Discs

All media used were prepared in accordance with manufacturers' instructions Simmons Citrate agar and MR–VP medium were products of Fluka Biochemical (Product of Spain). Antimicrobial discs used were Gram negative multo-discs containing following antimicrobials and content/disk: Ampicillin (PN: 30 µg), Augmentin (AU: 30 µg), Septrin (SXT: 30 µg), Ceporex (CEP: 10 µg), Peflacine (PEF: 10 µg), Streptomycin (S: 30 µg), Gentamicin (CN: 10 µg), Nalidixic acid (NA: 30 µg), Ofloxacin (OFX: 10 µg) and Ciprofloxacin (CPX: 10 µg).

Isolation of *Escherichia coli*

***Stool samples*:** A loop full of the stool sample was streaked on Sorbitol MacConkey (SMAC) agar (BIOTECH Laboratories Ltd., Ipswich, United Kingdom) and incubated for 24 hours at 37°C. Colorless (non-sorbitol fermenting) and pink (sorbitol fermenting) colonies were then sub-cultured on Eosine Methylene Blue (EMB) agar (BIOTECH Laboratories Ltd., Ipswich, United Kingdom) and incubated for 24 h at 37°C.

***Beef (raw and undercooked)*:** 1 g of the sliced beef sample was inoculated into 10 ml of Nutrient broth (NB) (BIOTECH Laboratories Ltd., Ipswich, United Kingdom) and incubated for 4 hours at 37°C. A loop full of the inoculum in nutrient broth was streaked on SMAC agar and incubated for 24 hours at 37°C. Colorless (non-sorbitol fermenting) and pink (sorbitol fermenting) colonies were then sub-cultured on EMB agar and incubated for 24 h at 37°C.

***Cabbage vegetable*:** 1 g of sliced vegetable sample inoculated into 10 ml of NB and incubated for 4 h at 37°C after which a loop full of the inoculum in the nutrient broth was streaked at SMAC agar and incubated for 24 hours at 37°C. Colorless (non-sorbitol fermenting) and pink (sorbitol fermenting) colonies were then sub-cultured on EMB agar and incubated for 24 h at 37°C.

"*Nono*": A loopful of the Nono sample was streaked on SMAC agar and incubated for 24 hours at 37°C. Colorless (non-sorbitol fermenting) and pink (sorbitol fermenting) colonies were then sub-cultured on EMB agar and incubated for 24 h at 37°C.

Identification of isolates

Identification of *E. coli* was as described by Cheesbrough (2006). Briefly, both sorbitol-fermenting and non-sorbitol fermenting colonies from SMAC that grew on EMB with metallic sheen characteristics were identified and confirmed as *E. coli* by microscopy (Gram staining) and minimal biochemical tests namely Indole (I), Methyl red (MR), Voges-Proskauer (VP) and Citrate (IMViC). Colonies that were Gram negative, indole positive, MR positive, VP negative and citrate negative were taken as *E. coli*. Such colonies were preserved on nutrient agar (NA) (BIOTECH Laboratories Ltd., Ipswich, United kingdom) slants at 4°C in a refrigerator; and reactivated by sub-culturing overnight on fresh NA at 37°C before use in experiments.

Antimicrobial Susceptibility Testing (AST)

Antimicrobial Susceptibility Testing was carried out by the Kirby-Bauer disc diffusion method in accordance with the United States' Clinical and Laboratory Standards Institute (CLSI) guideline (CLSI, 2007). Briefly three colonies of the isolated organism were sub-cultured into Mueller-Hinton broth (MHB) (BIOTECH Laboratories Ltd., Ipswich, United Kingdom) and incubated at 37°C for 24 h. MHB culture was streaked on the surface of sterile MHA plates using sterile cotton swabs. Multo antibiotic discs (Optun Laboratories Nig. Ltd.) were aseptically placed on the centre of inoculated Mueller-Hinton agar (MHA) (Fluka Biochemical, Product of Spain) plates and the plates were allowed to stand at room temperature for 20 min. The plates were then incubated at 37°C for 24 h. The zone of inhibition of the organism were measured (in mm) and recorded. *Escherichia coli* ATCC 25922 was used as an internal control. Interpretation of results was in line with CLSI criteria.

RESULTS

Isolation and Frequency of *Escherichia coli*

Escherichia coli was detected in 298 (69.3%) of the 430 samples as shown in Table 1. Of *E. coli* positive samples, 64 (21.5%) were non-sorbitol fermenting strains with high detection in raw beef (43.8%), cow dung (40.0%), cabbage vegetable (20.4%) and human stool (17.6%); 234 (78.5%) were sorbitol-fermenting strains with high detection in all sources.

Antimicrobial Susceptibility

The diameter of antibiotic zone of inhibition (in mm) of growth of non-sorbitol fermenting *E. coli* is as shown in Table 2; and the susceptibility of isolates to antibiotics is as summarized in Table 3.

Resistance Phenotypes

Antibiotic resistance phenotypes are as shown in Table 4 and summarized in Table 5. There are 46 different resistance phenotypes with AU, CPX, SXT, S, PN, CEP, OFX, NA, PEF; AU, CPX, PN, CEP, NA; AU, PN, CEP, NA, CN; PN, CEP, NA, CN; and CPX, PN, NA dominating at 4.7 % each.

Mu**ltiple Antibiotic Resistance (MAR) and MAR Index**

Multiple antibiotic resistance is defined here as joint resistance to at least 2 antibiotics. MAR was observed in all the isolates. Of the MAR isolates, resistance to 4 and 5 antibiotics were more frequent at 23.4% and 21.9%, respectively as shown in Table 4. MAR index above 0.2 implies that Isolates originates from environment where antibiotics are freely available with high potential for abuse or misuse (Krumpermann, 1983).

DISCUSSION

Escherichia coli which is a normal flora of the human and animal intestine has been identified as the leading cause of food borne illness all over the world (Hughes *et al.*, 2010). This study focused on the isolation and antimicrobial susceptibility of NSF *E. coli* from raw and undercooked beef, cabbage vegetable, "nono", stool samples from patients and cow dung. Although being documented by this study for the first time in Keffi, the isolation of NSF *E. coli* in raw beef, vegetables, "nono" (local milk product) and undercooked beef is not surprising as these sources are adjudged the common sources of this strain (Moses and Martinko, 2006; Elmali *et al.*, 2005; Solomon *et al.*, 2002; Agaolu *et al.*, 2000; Coker *et al.*, 2000). Fecal matter from cattle could be the most likely source of contamination of the beef as indicated by the high detection observed in cow dung in Keffi town. The high detection of NSF *E. coli* in cattle fecal samples agrees with earlier reports that cattle feces is the main reservoir of this strain (Callaway *et al.*, 2003, 2004). As most of the vegetables in Nigeria are grown with irrigated water that are in contact with grazing cattle (Solomon *et al.*, 2002), this can be a source of the observed contamination of the cabbage vegetables with NSF *E. coli* in this study. The observed presence of the strain in undercooked beef is in agreement with a reported outbreak of an unusual gastrointestinal following the consumption of contaminated hamburgers (Monero *et al.*, 2009; WHO, 2005).

The low susceptibility (i.e., higher resistance) of the isolates to the common and cheap orally administered antibiotics such as ampicillin, amoxicillin/clavulanic acid, nalidixic acid and cephalexin are not surprising because these drugs are more commonly misused; thereby leading to the development of resistance, as previously reported (Ehinmidu, 2003). The higher susceptibility of isolates to other antibiotics such as gentamicin, ofloxacin, and peflacin was expected, as this has been reported previously (Mbata, 2007). Gentamicin is administered parenterally and, therefore, due to the discomfort of injection, it is less likely to be misused than oral drugs. In contrast, ofloxacin and peflacine are relatively costly in Nigeria, and this limits their misuse. A MAR index > 0.2 indicates that bacteria originate from an environment where antibiotics are freely available and are misused (Krumpermann, 1983). This study provides important information on the best choice of antibiotics to treat infections that might arise from these organisms.

CONCLUSION

Non-sorbitol fermenting *E. coli* was isolated in Keffi metropolis from raw beef, cow dung, cabbage vegetables, human stool, undercooked beef and "nono" in order of decreasing frequency. The NSF *E. coli* were more susceptible to septrin, aminoglycosides and ofloxacin; originated from an environment where antimicrobials are freely available and misused; and were resistant to at least two of the antimicrobial agents tested. The presence of non-sorbitol fermenting *E. coli* in the food sources poses a potential risk of hazard to public health of humans considering the known danger associated with non-sorbitol fermenting *E. coli* infections, particularly in children. Public health education to improve kitchen practices and hygiene in farm and slaughter houses will help to reduce the contamination of beef during slaughter where cattle feces come in contact with the meat, ensure beef is properly cooked and vegetables are thoroughly washed before eating if it is to be eating fresh.

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Table 1: Isolation of sorbitol-fermenting and non-sorbitol fermenting *Escherichia coli* in Keffi, Nigeria.

Sample Source	**No. of samples examined**	**No. (%) of *E. coli* detected**	**No. (%) of sorbitol-fermenting *E. coli***	**No. (%) of non-sorbitol fermenting *E. coli***
Cabbage Vegetable	100	54 (54)	43 (79.6)	11 (20.4)
Raw beef	110	73 (66.4)	41 (56.2)	32 (43.8)
Undercooked Beef	60	27 (45)	26 (96.3)	1 (3.7)
"Nono"	50	49 (98)	48 (98.0)	1 (2.0)
Cow Dung	10	10 (100)	6 (60.0)	4 (40.0)
Stool (Human)	100	85 (18)	70 (82.4)	15 (17.6)
	430	**298 (69.3)**	**234 (78.5)**	**64 (21.5)**

Table 2: Diameter of zone of inhibition (mm) of non-sorbitol fermenting *Escherichia coli* isolated in Keffi, Nigeria for commonly used antimicrobial agents.

Isolates	AU			CPX			SXT			S			PN			CEP			OFX			NA			PEF			CN		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
EC-1-V			13			15			10			6			0			10			12			13			7		14	
EC-2-V		16		22			21				13			14		20					0		14				12			9
EC-3-V			10			10		11			12				12			14	19					12	17			19		
EC-4-V			12			15	17					10			8			0	18					0		16		15		
EC-5-V		17			16		18					10			11	19					0	20					10	15		
EC-6-V			8		18			12			13				8			13	20			20			19			18		
EC-7-V		17			16		15			18					10			11	19					0	20					10
EC-8-V			11		19		18			17					0			0	20					0	18					12
EC-9-V			13			15			10			6			0			10			12			7			12		14	
EC-10-V			10			13			10		12				12			14	19					12	17			19		
EC-11-V			13			15			10			6			0			10			12			7			12		14	
EC-12-Rb			8		17			12				6			9			13	20			18			18					9
EC-13-Rb			11		19		18				12				0			0	20					0	18					12
EC-14-R**b**		15			20				0	15					13		16		22					0	21				13	
EC-15-Rb			12			15	17			16					8			0	18					0		16		15		
EC-I6-Rb			13			13			0			10			11			0	19					0	18					9
EC-17-Rb			8		17			12			14			16		22				13		18				16				8
EC-18-Rb		15			17			12			13				0			14	20				16		18				13	
EC-19-Rb			11		20		16			15					9			12	18				16			14				9
EC-20-Rb			13			15			10	17					0			10			12			7			12		14	
EC-21-Rb			10			13		11				10			12			14	19					12	17			19		
EC-22-Rb		15				12		14		16					10		15		21					0	20				14	
EC-23-Rb			14	21				13			12				0			14	20				14		24			17		
EC-24-Rb		15			17			12			13			16		22			19			19					9			8
EC-25-Rb			13			15			10	17					0			10			12			7			12		14	
EC-26-Rb			10			13		11			14				13			14	19					12	17			19		
EC-27-Rb		15				12		14				10	17					9			12	20				14		19		
EC-28-Rb			12		18		18				12				13		16		20					0	22				14	
EC-29-Rb			12			15	17					0			8			0	17			19					12	15		
EC-30-Rb			8			9		12				10			12	19					11	19					12			9
EC-31-Rb			12			14	29			16			19					0		14		21			20				14	
EC-32-Rb		15				12	19					6	21					0	20				14		19				14	
EC-33-Rb			11		19		18				12				0			13			11	20					11			12
EC-34-Rb			12			15	17			19					8			0	17			19					9	15		
EC-35-Rb			12		18		18			19					13		16		20					0	22				14	
EC-36-Rb			10			13		12				0			14	19					12		17		19					9
EC-37-Rb		15				12		14		18					11		0				0			9		15			14	
EC-38-Rb			11		19		18			17					0			12	20					0	18					12
EC-39-Rb			8		18			12			13				12	19				13				13			12	19		
EC-40-Rb			12			15	17					10			0			0	18				14			15				9
EC-41-Rb			12		19		18			15					0			0			12	19			20			19		
EC-42-Rb			11		19		18			17					0			0	20					0	18			20		
EC-43-Rb			8			12	18				13				9			13	20			18			17					9
EC-44-Rb		15			19				0		14				11			14			2			0	21				14	
EC-45-N		15			20				0		14				12		16		22		0	21				12			13	
EC-46-Cd			13	21			17			18					0		17				12			0	18					10
EC-47-Cd		17		21				13		19					0			14	20				14		24			17		
EC-48-Cd		15				12		12		19					10		15		21					0	20				14	
EC-49-Cd		17			16		16			18					10			11	19					0	20					10
EC-50-St		17		21				13		19					0			14	20				14		24			17		
EC-51-St		15				12		14		19					10		15		21					0	20				14	
EC-52-St			0			0		12		18					10			14	21				17		18					9
EC-53-St		17			16		15			18					10			11	19					0	20					10
EC-54-St			11		19		18			17					0	20				14				9			12			11

EC –*Escherichia coli*; V – Vegetable; Rb – Raw Beef; N–"Nono"; Cd–Cow Dung; St–Human Stool; S – susceptibility; I – Intermediate; R – resistance

Table 2 Continued

Isolates	AU			CPX			SXT			S			PN			CEP			OFX			NA			PEF			CN		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
EC-55-St			12			14	15					11			9			9	19				14				12	20		
EC-56St			12			15	19		0			10			8			0	18					0		16			15	
EC-57-St			0			10		11		18					13			13		16				0			9			9
EC-58-St			10			13	20				12				12			14	19					12	19					12
EC-59-St			12			14		14				9			12			13		14		19					9			9
EC-60-St		15				12		14		19				15		21					0	20					9		14	
EC-61-St		16			18		18				13				12	20			21					0		19			13	
EC-62-St			12		18				0	16					13		16		20					0	22				14	
EC-63-St		14				13		14		16					0			0			10			0			13	18		
EC-64-St			13	22					10			6		16			15				11		12				12			10

EC –*Escherichia coli*; St–Human Stool; S – susceptibility; I – Intermediate; R – resistance; EC – *Escherichia coli*; V – Vegetable; Rb – Raw Beef; S – susceptibility; I – Intermediate; R – resistance.

Table 3: Susceptibility to common antimicrobial agents of non-sorbitol fermenting *Escherichia coli* isolated in Keffi, Nigeria

Antimicrobial Agents	Disc Content (µg)	No. (%) Susceptible (n = 64)	No. (%) Resistant (n = 64)
Septrin (SXT)	30	51 (79.7)	13 (21.3)
Ampicilin (PN)	30	8 (12.5)	56 (87.5)
Augmentin (AU)	30	21 (32.8)	43 (67.2)
Ceporex (CEP)	10	21 (32.8)	43 (67.2)
Peflacine (PEF)	10	44 (68.8)	20 (31.2)
Streptomycin (S)	30	46 (71.9)	18 (28.1)
Gentamicin (CN)	10	40 (62.5)	24 (37.5)
Nalidixic acid (NA)	30	28 (43.8)	36 (56.2)
Ofloxacin (OFX)	10	45 (70.3)	19 (29.7)
Ciprofloxacin (CPX)	10	31 (48.4)	33 (51.6)

Table 4: Antimicrobial resistance profiles of non-sorbitol fermenting *Escherichia coli* isolated in Keffi, Nigeria.

Isolates	Resistance Profile									
EC-1-V	AU	CPX	SXT	S	PN	CEP	OFX	NA	PEF	
EC-2-V							OFX		PEF	CN
EC-3-V	AU	CPX			PN	CEP		NA		
EC-4-V	AU	CPX		S	PN	CEP		NA		
EC-5-V				S	PN		OFX		PEF	
EC-6-V	AU				PN	CEP				
EC-7-V					PN	CEP		NA		CN
EC-8-V	AU				PN	CEP		NA		CN
EC-9-V	AU	CPX	SXT	S	PN	CEP	OFX	NA	PEF	
EC-10-V	AU	CPX	SXT		PN	CEP		NA		
EC-11-V	AU	CPX	SXT	S	PN	CEP	OFX	NA	PEF	
EC-12-Rb	AU			S	PN	CEP				CN
EC-13-Rb	AU				PN	CEP		NA		CN
EC-14-Rb			SXT		PN			NA		
EC-15-Rb	AU	CPX			PN	CEP		NA		
EC-I6-Rb	AU	CPX	SXT	S	PN	CEP		NA		CN
EC-17-Rb	AU									CN
EC-18-Rb					PN	CEP				
EC-19-Rb	AU				PN	CEP				CN
EC-20-Rb	AU	CPX	SXT		PN	CEP	OFX	NA	PEF	
EC-21-Rb	AU	CPX		S	PN	CEP		NA		
EC-22-Rb		CPX			PN			NA		
EC-23-Rb	AU				PN	CEP			PEF	CN
EC-24-Rb									PEF	CN
EC-25-Rb	AU	CPX	SXT		PN	CEP	OFX	NA	PEF	
EC-26-Rb	AU	CPX			PN	CEP		NA		
EC-27-Rb		CPX		S		CEP	OFX			
EC-28-Rb	AU				PN			NA		
EC-29-Rb	AU	CPX		S	PN	CEP			PEF	
EC-30-Rb	AU	CPX		S	PN		OFX		PEF	CN
EC-31-Rb	AU	CPX				CEP				
EC-32-Rb		CPX		S		CEP				
EC-33-Rb	AU				PN	CEP	OFX		PEF	CN
EC-34-Rb	AU	CPX			PN	CEP			PEF	
EC-35-Rb	AU				PN			NA		
EC-36-Rb	AU	CPX		S	PN		OFX			CN
EC-37-Rb		CPX			PN	CEP	OFX	NA		
EC-38-Rb	AU				PN	CEP		NA		CN
EC-39-Rb	AU				PN			NA	PEF	
EC-40-Rb	AU	CPX		S	PN	CEP				CN
EC-41-Rb	AU				PN	CEP	OFX			
EC-42-Rb	AU				PN	CEP		NA		

EC = *Escherichia coli*; V = Vegetable; Rb = Raw Beef

Table 4 Continued

Isolates	Resistance Profile									
EC-43-Rb	AU	CPX			PN	CEP				CN
EC-44-Rb			SXT		PN	CEP	OFX	NA		
EC-45-N			SXT		PN		OFX			
EC-46-Cd	AU				PN		OFX	NA		CN
EC-47-Cd					PN	CEP				
EC-48-Cd		CPX			PN			NA		
EC-49-Cd					PN	CEP		NA		CN
EC-50-St					PN	CEP				
EC-51-St		CPX			PN			NA		
EC-52-St	AU	CPX			PN	CEP				CN
EC-53-St					PN	CEP		NA		CN
EC-54-St	AU				PN			NA	PEF	CN
EC-55-St	AU	CPX		S	PN	CEP			PEF	
EC-56-St	AU	CPX	SXT	S	PN	CEP		NA		
EC-57-St	AU	CPX			PN	CEP		NA	PEF	CN
EC-58-St	AU	CPX			PN	CEP		NA		CN
EC-59-St	AU	CPX		S	PN	CEP			PEF	CN
EC-60-St		CPX					OFX		PEF	
EC-61-St					PN			NA		
EC-62-St	AU		SXT		PN			NA		
EC-63-St		CPX			PN	CEP	OFX	NA	PEF	
EC-64-St	AU		SXT	S			OFX		PEF	CN

EC = *Escherichia coli*; Rb = Raw Beef; N = “Nono”; Cd = Cow Dung; St = Human Stool

Table 5: Antibiotic resistance phenotypes of non-sorbitol fermenting *Escherichia coli* isolated in Keffi, Nigeria.

Resistance Phenotypes	Number of isolates with the pattern	Frequency (%) (n = 64)
AU, CPX, SXT, S, PN, CEP, OFX, NA, PEF	3	4.7
AU, CPX, SXT, PN, CEP, OFX, NA, PEF	2	3.1
AU, CPX, SXT, S, PN, CEP, NA, CN	1	1.6
AU, CPX, S, PN, OFX, PEF, CN	1	1.6
AU, CPX, SXT, S, PN, CEP, NA	1	1.6
AU, CPX, PN, CEP, NA, PEF, CN	1	1.6
AU, CPX, S, PN, CEP, PEF, CN	1	1.6
AU, CPX, S, PN, CEP, NA	2	3.1
AU, CPX, SXT, PN, CEP, NA	1	1.6
AU, CPX, S, PN, CEP, PEF	2	3.1
AU, PN, CEP, OFX, PEF, CN	1	1.6
AU, CPX, S, PN, OFX, CN	1	1.6
AU, CPX, S, PN, CEP, CN	1	1.6
AU, CPX, PN, CEP, NA, CN	1	1.6
AU, SXT, S, OFX, PEF, CN	1	1.6
CPX, PN, CEP, OFX, NA, PEF	1	1.6
AU, CPX, PN, CEP, NA	3	4.7
AU, PN, CEP, NA, CN	3	4.7
AU, PN, CEP, PEF, CN	1	1.6
AU, CPX, PN, CEP, PEF	1	1.6
AU, CPX, PN, CEP, CN	2	3.1
AU, S, PN, CEP, CN	1	1.6
AU, PN, NA, PEF, CN	1	1.6
AU, PN, OFX, NA, CN	1	1.6
CPX, PN, CEP, OFX, NA	1	1.6
SXT, PN, CEP, OFX, NA	1	1.6
AU, SXT, PN, NA	1	1.6
AU, PN, CEP, OFX	1	1.6
AU, PN, CEP, NA	2	3.1
AU, PN, CEP, CN	1	1.6
AU, PN, NA, PEF	1	1.6
CPX, S, CEP, OFX	1	1.6
S, PN, OFX, PEF	1	1.6
PN, CEP, NA, CN	3	4.7
AU, CPX, CEP	1	1.6
AU, PN, NA	2	3.1
CPX, S, CEP	1	1.6
CPX, PN, NA	3	4.7
CPX, OFX, PEF	1	1.6
SXT, PN, OFX	1	1.6
SXT, PN, NA	1	1.6

SXT = Septrin; AU = Augmentin; PN = Ampicillin; CPX = Ciprofloxacin; NA = Nalidixic acid; CEP = Ceporex; CN = Gentamicin; S = Streptomycin; OFX = Ofloxacin; PEF = Peflacine.

Table 5 Continued

Resistance Phenotypes	Number of isolates with the pattern	Frequency (%) (n = 64)
OFX, PEF, CN	1	1.6
AU, CN	1	1.6
PN, CEP	3	4.7
PN, NA	1	1.6
PEF, CN	1	1.6

SXT = Septrin; AU = Augmentin; PN = Ampicillin; CPX = Ciprofloxacin; NA = Nalidixic acid; CEP = Ceporex; CN = Gentamicin; S = Streptomycin; OFX = Ofloxacin; PEF = Peflacine.

Table 6: Frequency of multiple antibiotic resistances and multiple antibiotic resistance indices of non-sorbitol fermenting *Escherichia coli* isolated in Keffi, Nigeria.

Number of antibiotics resistance to	Number of isolates with resistance to (a)	Number of antibiotics tested (b)	Percentage of MAR isolates	MAR index (a/b)
9	3	10	4.7	0.3
8	3	10	4.7	0.3
7	4	10	6.3	0.4
6	11	10	17.2	1.1
5	15	10	23.4	1.5
4	14	10	21.9	1.4
3	11	10	17.2	1.1
2	6	10	9.4	0.6