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Adaeze Joy Alu[a], Gabriel K. Omeiza[a], James A. Ameh[b], Enem S.I[b]

[a] Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Abuja, F.C.T., Nigeria.
[b] Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Abuja, F.C.T., Nigeria.

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Prevalence and antibiotic resistance profile of Shiga-toxigenic *Escherichia coli* O157 (STEC) from retailed miscellaneous meat and fish types in Abuja, Nigeria

Adaeze Joy Alu*[a], Gabriel K. Omeiza[a], James A. Ameh[b], Enem S.I[b]

[a] Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Abuja, F.C.T., Nigeria.
[b] Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Abuja, F.C.T., Nigeria.
Emails: [a] adaeze.alu@uniabuja.edu.ng, [b] gabriel.omeiza@uniabuja.edu.ng, [c] jamesameh10@gmail.com

*** Corresponding author:** Alu, AJ., E-mail: adaeze.alu@uniabuja.edu.ng

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Abstract

Most *Escherichia coli* strains are harmless intestinal bacteria of animals, but some are implicated in food infection/poisoning especially Shiga toxin (or Vero toxin) producing *E. coli* (STEC) due to consumption of meat. This study was conducted to determine the prevalence and antibiotic resistance profile of Shigatoxigenic *Escherichia coli* O157 (STEC) from retailed miscellaneous fish and meat types in Abuja, Federal Capital Territory, Nigeria. A total of 256 meat and fish consisting of cow muscles, intestines, rumen-sacs, livers and tails, cat-fish, frozen fish (mackerel and herrings) were examined. *Escherichia coli* were isolated by enrichment culture cefixime-tellurite sorbitol MacConkey agar (CT-SMAC), morphological, biochemical, serotype latex agglutination and disk diffusion methods. Of the 256 samples, 138 (53.9%) were contaminated with *E. coli* and 28 (21.7%) *E. coli* strains were positive for Shigatoxigenic *Escherichia coli* O157 (STEC). Meat muscles had the highest prevalence of STEC (7.41%) among meat samples, followed by rumen-sacs (6.0%), intestines (5.77%), tails (4.0%), and the prevalence of STEC in Fish includes Cat-fish intestine (26.7%), skin (21.4%), Mackerel intestine (26.7%), skin (14.3%), and Herrings skin (15.4%), gill (7.1%). All the STEC assessed indicated multi-drug resistance, with the isolates showing 100% resistant to ampicilin, and erythromycin, nitrofurantoin (95.7%), amoxicilin clavulanic acid (84.3%), sulphamethaxazole/trimethoprim (75%), streptomycin (75%), tetracycline (66.17%), and gentamycin (53.6%). The isolates were susceptible to ciprofloxacin (66.7%), Cefoxitin (66.7%), amikacin (39.3%), and chloramphenicol (35.7%). The implication of STEC in this study suggests that contaminated meat types are sold to consumers and can result to serious foodborne hazards. Prescription of ciprofloxacin and cefoxicilin are recommended against this organism. Application of good hygienic procedures in meat and fish handling processes and proper boiling before consumption can mitigate the risk of infection due to resistance STEC strains.

Keywords: *Serotyping; Latex agglutination; Antibiotic-resistance; Escherichia coli.*

1. Introduction

Escherichia coli a facultative anaerobe usually found in the gastrointestinal tract of mammals, belonging to the family Enterobacteriaceae, (CDC, 2012). The emergence of O157 strains of *Escherichia coli* poses serious threat to public health with regards to their devastating and zoonotic importance, (Wasteson, 2001; Alexis et al. 2010). *Escherichia coli* O157:H7 is a highly virulent Enterohemorrhagic *Escherichia coli* with an acronym (EHEC) used to denote a sub-set of Shiga toxin (Stx)-producing *E. coli* (STEC) also recognized as vero toxin producing *E. coli* (VTEC), which causes serious disease conditions in humans including hemorrhagic colitis, bloody diarrhea and the hemolytic uremic syndrome (HUS), (Wasteson, 2001). *E. coli* O157:H7 was first isolated from hamburger and cattle as reported by the Centers for Disease Control (CDC 1982) which are considered to be the prominent reservoir for EHEC O157. *E. coli O157* is commonly found in the intestines of ruminants and cross contamination of any parts of the animal is possible when meat processing is not properly done, ruminants are usually exposed to STEC due to contaminated feed and drinking water as well as exposure to the feces of other animals shedding the bacteria (LeJeune et al. 2001; Persad and LeJeune 2014). The bacterium (*E. coli* O157) is also found naturally in the intestines of other animals like pigs, sheep, goats and deer as well as in milk, vegetables and fish (Govindarajan, 1990; WHO, 2004; Thampuran et al. 2005; Yousuf, 2008; Shafiullah et al. 2018; Yakubu, 2018). Few studies have evaluated antimicrobial resistance of *E. coli* O157 in Sub-Saharan Africa. Shigatoxin-producing *E. coli* O157 has become a major meat safety issue worldwide, (Nakazawa et al. 1999; Okeke et al. 2000).

Meat and fish are major staple in Nigerian food, according to government estimates, Nigeria, consumes beef in tone of over 360,000 tunes each year, accounting for half of all West Africa, with emerging middle-class population of over 200 million people. There is a booming demand for meat and fish in Nigeria, consumption of meat and fish is low compared with advanced economies in per-capita terms, but growing fast in alarming rate and expected to quadruple by 2050, (FAO, 2017). Unwholesome meat can constitute great degree of health hazard to its consumer, especially when implicated with Shiga-toxin producing *E. coli* strains, (WHO, 2018). There is dart of information on these virulent and resistance strain of *E. coli* from meat and fish in Abuja. This paper, therefore, reports on the prevalence and antibiotic profile of Shigatoxigenic *Escherichia coli* O157 (STEC) from meat and fish in Abuja, Nigeria, to highlight the potential threat to public health and safety.

2. Materials and Methods

Area/Sources and collection of samples:

This study was carried out in Abuja, Federal Capital Territory (FCT), which is made up of six Area councils. Geographically, F.C.T. is placed at the latitude 9.0578499 and longitude 7.49508, in the northern hemisphere. Sharing borders with Niger state to the west/north, Kaduna state to the north/east, Nasarawa state to the east/south and Kogi state to the south/west, covering a landmass of approximately 7,315 km^2, with moderate climatic conditions, having estimated population of 1,406,239 as at the 2006 population census (NPC, 2006). The total of 256 meat (n= 128) and fish (n=128) samples was used for this study, collected from local markets in three area councils of F.C.T. randomly selected, namely; Kwali, Gwagwalada, and Bwari Area Councils. All meat and fish samples were bought in wraps as they would normally be sold to the consumer from the various markets and were appropriately labeled, placed in a flask with ice, then transported immediately for analysis in the laboratory between 2 to 6 hours after collection.

Bacteriological examination:

About 10 g of samples each were stomached and diluted in 90 ml of peptone water (Merck), then cultured overnight at 37°C. Prepared sample broth culture was plated onto MacConkey agar, Eosin methylene blue agar (EMB) (Oxoid) for the identification of the green metallic sheen morphological characteristics of *E. coli* colonies. The colonies were further inoculated into Sorbitol MacConkey agar (Oxoid) enriched with cefixime tellurite supplement (CT-SMAC) (Oxoid) to selectively distinguish the non-sorbitol fermenting *E. coli* O157 strains from other *E. coli* strains isolated, each sample was streaked onto the media surface and incubated at 37°C for 24 hours (Janet et al. 2003). The morphological characteristics, sorbitol fermentation, gram staining and motility of the colonies were tested. Biochemical tests on the presumptive *E. coli* colonies were performed and the isolates were identified according to standard methods, (Cheesbrough, 2006), then stored in the refrigerator at 4°C on slants of nutrient agar for further work.

Serotyping of the Isolates:

Characterization of the STEC serotype was done by slide agglutination with antisera according to the method of Nataro and Kaper (1998). The shigatoxigenic *E. coli* O157 antisera rapid latex agglutination test kit (Oxoid) was used to serotype *E. coli* strains. With drops of antisera on slide trays with wells, colonies were examined for the shigatoxigenic *E. coli* O157. The isolates were tested using the control latex reagents for nonspecific agglutination of organisms with latex. Positively reactive O157 colonies were transferred to other

slant medium for further testing, which allows for more yield of the bacterial growth on which to perform more O157 agglutination assay. Strains that agglutinate with latex reagents were considered as *E. coli* O157 serotype, (Blanco et al. 2003).

Antibiotic Resistance testing:

Antimicrobial susceptibility testing of the *E. coli* O157 isolates to different antimicrobial agents was performed according to the Clinical and laboratory standards institute guidelines and the agar disk diffusion method (CLSI, 2015; Bauer et al. 1966), with 12 commercially available antimicrobial agents (Oxiod) on Mueller-hinton agar (USA), which includes ciprofloxacin (CIP) 5 µg, erythromycin (E) 15 µg, ampicillin (AMP) 10 µg, amoxicillin/calvulanic acid (AMC) 30 µg, sulphamethoxazole/trimethoprim (SXT) 25 µg, cefoxitin (FOX) 30 µg, tetracycline (TE) 30 µg, amikacin (AK) 30 µg, streptomycin (S) 10 µg, nitrofurantoin (F) 300 µg, chloramphenicol (C) 30 µg, and gentamicin (CN) 10 µg. The agar plates were prepared according to the manufacturer guidelines, with a sterile glass spreader, broth culture was spread gently over the surface of agar plates and allowed to dry for 5 min. The antibiotic discs were firmly placed on the agar surface with 1 cm distance apart and incubated at 37°C for 20 hours. The diameter of inhibition zone formed around each disc was measured and evaluated according to CLSI (2015).

Statistical Analysis:

Chi square analysis (in Statistical package for social sciences; version 20.0) was used to test associations in means of different retailed meat and fish types with locations where they were sampled at 95% CI, such that values less than 0.05 ($P < 0.05$) was considered significant.

3. Results

Detection of *E. coli*: Table 1; shows the prevalence of *E. coli* in various types of meat and fish studied. From the results, 58.59% (n=75/128) meat and 49.21% (n=63/128) fish samples harbored *E. coli* strains, totaling 53.9% (n=138/256) meat and fish samples. There were statistically significant differences observed in the frequency of *E. coli* in various samples ($P < 0.05$).

Detection of STEC: Table 2 and 3; characterizes the prevalence of shigatoxigenic *E. coli* O157 in meat and fish samples with overall prevalence of 10.9% (n=28/256), out of 138 *E. coli* isolates 20.3% (n=28/138) strains were characterized and confirmed as *E. coli* O157 serotypes, with the prevalence of 9.4% (n=12/128) in meat and12.5% (n=16/128) from fish. Frequency of *E. coli* O157 cluster in various Meat types includes cow meat muscles (11.5%), intestine (15.4%), rumen sac (11.5%), tail muscles (4.0%), and liver (0%), while, O157 serotype in Fish types includes Cat-fish intestine (26.6%), skin (21.4%), gill (0%), and muscles (0%), Mackerel intestine (14.2%), skin (14.2%), gill (14.2%), and muscles (0%), and Herrings skin (14.2%), gill (7.14%), intestine (0%), and muscles (0%). *E. coli* O157 was implicated in some meat and fish studied with statistically significant differences observed ($P < 0.05$).

Table (1): Meat and fish types showing prevalence of *E. coli*

Crosstab		E. coli		E. coli O157:H7	Total No
Sample		NEG	POS (%)	POS (%)	
Meat Type	Cow Liver	16	9 (36.0)	0	25
	Cow tail	8	17(68.0)	2(8.0)	25
	Cow Muscle	8	18(69.2)	3(11.5)	26
	Cow Rumen-sac	15	11(42.3)	3(11.5)	26
	Cow Intestine	6	20(76.9)	4(15.4)	26
Total		**53**	**75(58.6)**	**12(9.4)**	**128**
Fish Type	Herring Skin	10	4 (28.5)	2(14.2)	14
	Herring Gill	7	7(50.0)	1(7.14)	14
	Herring Intestine	3	12(80.0)	0	15
	Mackerel Skin	10	4(28.6)	2(14.2)	14
	Mackerel Gill	6	8(57.1)	2(14.2)	14
	Mackerel Intestine	7	7(50.0)	2(14.2)	14
	Catfish Skin	8	6(42.9)	3(21.4)	14
	Catfish Gill	11	3(21.4)	0	14
	Catfish Intestine	3	12(80.0)	4(26.6)	15
Total		**65**	**63(49.2)**	**16(12.5)**	**128**
Overall Total (Meat & Fish)		**118**	**138(53.9)**	**28(10.9)**	**256**

Table (2): Sample Meat & Fish * E. coli O157:H7 Cross tabulation

			E. coli O157:H7		
			NEG	POS	Total
Sample	Meat	Count	116	12	128
		Expected Count	114.0	14.0	128.0
	Fish	Count	112	16	128
		Expected Count	114.0	14.0	128.0
Total		Count	228	28	256
		Expected Count	228.0	28.0	256.0

Chi-Square Tests	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	.642[a]	1	.423
Continuity Correction[b]	.361	1	.548
Likelihood Ratio	.644	1	.422
Fisher's Exact Test			
Linear-by-Linear Association	.639	1	.424
N of Valid Cases	256		

The calculated value of Chi-square is 0.642 and the P-value is 0.423 which is greater than the level of significance of 0.05(5%), we accept the null hypothesis and conclude that the level of contamination due to *E. coli* O157 in fish is not statistically different from that of beef.

$H_0$1: The level of contamination due to E. coli O157 in fish is not statistically different from that of beef.

Table (3): $H_0$2: No statistically significant different observed in the level of contamination due to *E. coli* O157 among the different types of fish used for the study.

Fish Types * *E. coli* O157 Cross tabulation

			E. coli O157		
			NEG	POS	Total
Fish Type	Herring Skin	Count	12	2	14
		Expected Count	12.3	1.8	14.0
	Herring Gill	Count	13	1	14
		Expected Count	12.3	1.8	14.0
	Herring Intestine	Count	15	0	15
		Expected Count	13.1	1.9	15.0
	Mackerel Skin	Count	12	2	14
		Expected Count	12.3	1.8	14.0
	Mackerel Gill	Count	12	2	14
		Expected Count	12.3	1.8	14.0
	Mackerel Intestine	Count	12	2	14
		Expected Count	12.3	1.8	14.0
	Catfish Skin	Count	11	3	14
		Expected Count	12.3	1.8	14.0
	Catfish Gill	Count	14	0	14
		Expected Count	12.3	1.8	14.0
	Catfish Intestine	Count	11	4	15
		Expected Count	13.1	1.9	15.0
Total		Count	112	16	128
		Expected Count	112.0	16.0	128.0

Chi-Square Tests	Value	Df	Asymptotic Significance (2-sided)
Pearson Chi-Square	8.446[a]	8	.391
Likelihood Ratio	11.370	8	.182
Linear-by-Linear Association	1.219	1	.270
N of Valid Cases	128		

a. 9 cells (50.0%) have expected count less than 5. The minimum expected count is 1.75

The calculated value of Chi-square is 8.446 and the P-value is 0.391 being greater than the level of significance of 0.05(5%), we therefore accept the null hypothesis and conclude that there is no statistically significant difference in the levels of contamination due to *E. coli* O157 among the different type of fish used for the study.

Antibiotic Sensitivity: Figure 1; highlights the multidrug resistance pattern of *E. coli* O157 in this study. The *E. coli* isolates exhibited 100% resistant to ampicilin, and erythromycin, nitrofurantoin (95.7%), amoxicilin clavulanic acid (84.3%), sulphamethaxazole/trimethoprim (75%), streptomycin (75%), tetracycline (66.17%), and gentamycin (53.6%). The isolates were susceptible to ciprofloxacin (66.7%), cefoxitin (66.7%), amikacin (39.3%), and chloramphenicol (35.7%). *See* Figure 1.

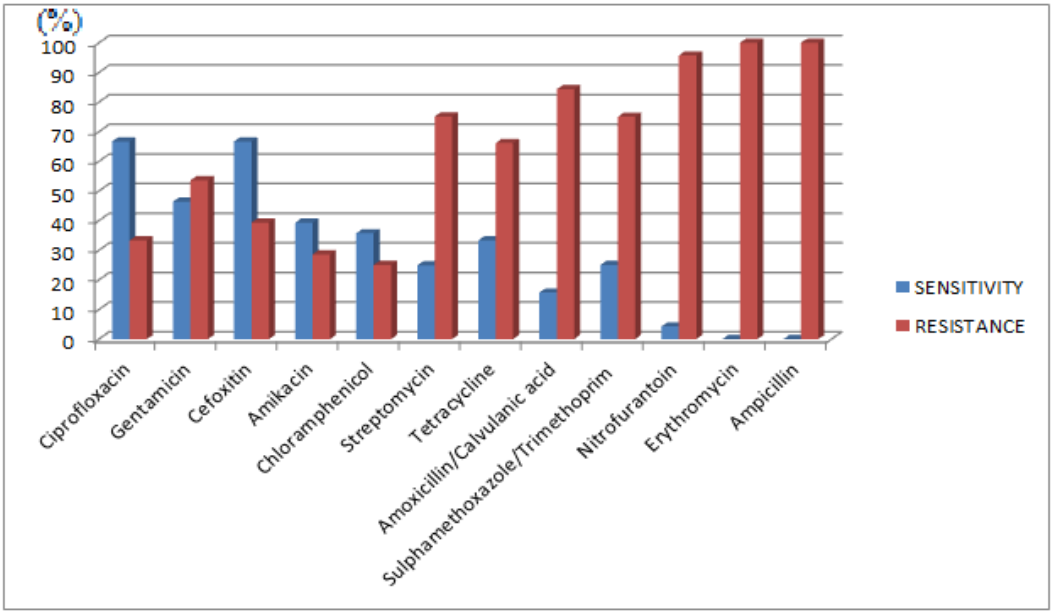

Figure (1): Percentage distribution of resistance pattern of *E. coli* O157 isolates to tested antibiotics

4. Discussion

The prevalence of STEC O157:H7 in animal and food products from almost all over Africa poses great risk for human infection (Athumani, 2017); the current surveillance system worldwide reveals the diversity and impact of STEC infections as well as sources of contamination. High increase in STEC outbreaks over the past 10 years due to contaminated food and contact with animals and animal products is a public health concern, (Jun-Seob, 2020). The need to understand animals and food sources as potential reservoir for STEC is quite essential.

Of the 256 meat and fish studied, results show prevalence rate of 10.9% (28) STEC strains, among these number 12 (9.4%) were from meat and 16 (12.5%) from fish. This is in agreement with similar work done by Kumar (2004) on seafood and beef in Mangalore. Among these number 12 (9.4%) were from meat and 16 (12.5%) from fish. Also Ameh et al. (2002), isolated shigatoxigenic *E. coli* O157 from both diarrheic infants and calves from Maiduguri, at the prevalence of 10.53%, where they reported that the prevalence declined with increase in age.

In this study, the prevalence of STEC in meat agrees with 10% STEC prevalence from beef and rectal swabs reported by Shafiullah et al. (2018) in Bangladesh, but higher than the 3.5% prevalence in ground beef reported by Alzira et al. (2007), however contrary to this work, Mashak (2018) reported higher prevalence of 14% in raw meat.

We recorded 12.5% prevalence of STEC in various types of fresh fish and frozen fish from retail markets, these is contrary to similar work done by Thampuran et al. (2013), who worked on 23 different types of fresh and frozen fish from retail markets, reporting *E. coli* O157 strain to be absent but had the presence of other MUG and sorbital-negative and virulent types of *E. coli* suggesting for further studies in fish. Also, Kumar et al. (2010) screened *E.coli* isolated from fish, clams, and water for specific genes of stx, hlyA and rfbO157; He reported that 5% clam and 3% fresh fish samples were positive for non-O157 STEC. However, this study agrees with the work done by Gupta et al. (2013), who isolated STEC from raw fish and fish products from retail markets of the Ludhiana, purporting raw fish to be the major source for virulence gene of STEC. Similarly, Surendraraj et al. (2010) reported significant incidence of shigatoxigenic *Escherichia coli* in fish and shrimp from different retail fish markets in Cochin. Generally there is paucity of literature on STEC in fish in this region.

The antimicrobial resistance pattern in this study revealed that there is multiple drug resistance of the isolates to most of the antibiotics used, but was mostly susceptible to ciprofloxacin and cefoxitin. Globally, antimicrobial resistances are now observed in high frequencies, (Mukherjee et al. 2017), several studies implicated *stx1* gene as encoding antimicrobial (AMR) in STEC O157.

5. Conclusion

This study provides evidence of contamination of common street vented edible meat and fish types circulating in local markets of the Area councils constituting the Federal Capital Territory. Documented prevalence of *E. coli* varied amongst the different sampled area councils.

This study provides evidence that toxigenic *Escherichia coli* O157 strains are also common contaminants of meat and fish types in selected Area councils of Abuja. The antibiotic resistance strain of *E. coli* O157 found in this study are of public health importance, therefore, there is urgent need to

legislate to assure compliance on hygiene on street vended meat and fish types in the FCT.

There is need for public awareness on the epidemiology of the pathogen. Appropriate hygienic measures must be imbibed by handlers of meat and fish products. Proper cooking of meat and fish before consumption is highly recommended.

This study suggests a wide collaborative study of all street retailed meat and fish products to understand sources of food contaminants and policies to mitigate them.

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