

Isolation, Identification and Antimicrobial Susceptibility of Shiga Toxin-Producing *Escherichia coli* Serotypes in Chickens in Maiduguri, Nigeria

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Abstract: This study was carried out in Maiduguri to isolate, characterize and determine the antimicrobial susceptibility of Shiga Toxin-producing *Escherichia coli* (STEC) in broilers and village chickens faeces. Conventional microbiology culture, phenotypical characterization, and biochemical test were used. Three hundred (300) (150 each of broiler and village chickens) faecal samples were randomly collected from chickens brought to the Maiduguri Monday Market Chicken slaughterhouse. A total of 83 (27.7%) *E. coli* isolates were obtained comprising 36(24%) from broiler chickens and 47(31.3%) from village chickens. The *E. coli* positive isolates were then serotyped based on their somatic 'O' antigen using latex agglutination test for O157 STEC and dry spot polyvalent Sero-check for Non-O157 STEC. Total of 19 (12.7%) and 26 (17.3%) were O157 STEC; while 8(5.3%) and 9(6%) were non-O157 STEC for both broilers and village chickens respectively. The remaining *E. coli* isolates 9(6%) from broiler chickens, and 12(8%) from village chickens were untypable using the conventional sero kits. The result of the antimicrobial sensitivity test using Clinical and Laboratory Standard Institute Guide revealed that the STEC isolates were susceptible to the antibiotics. With high susceptibility to Ciprofloxacin (100%), Ceftriaxone (98%) and Gentamicin (100%) and low susceptibility to Trimethoprim (79%), Chloramphenicol (66.6%) and resistant to cefodizime (73.1%) and Ampicillin (75%).

Keywords: Broiler chickens, Village chickens, Shiga toxin-producing *E. coli*, stx1, stx2, O157, non O157, antimicrobial susceptibility.

I. INTRODUCTION

Escherichia coli (*E. coli*) is a group of bacteria whose members are typically non-pathogenic and are normal microflora of the intestinal tract of humans and animals (Gyles, 2006). However,

some of these bacterial species have acquired genes that enable them to cause intestinal disease. The *E. coli* that cause enteric diseases divided into prototype based on their virulence factors and mechanism by which they cause the disease. One of these pathogens, called Shiga toxin-producing *E. coli* refers to those strains of *E. coli* that produce at least one member of a class of potent cytotoxin called Shiga toxins (Gyles, 2006). During the past two decades, an increasing number of food-borne illness outbreaks have traced to consumption of undercooked ground beef and other beef products contaminated with Shiga toxin-producing *E. coli* O157. Shiga toxin *E. coli* also refers to as Verocytotoxin-producing *E. coli* (VTEC). These STEC or VTEC are the causes of major, potentially fatal zoonotic food-borne illnesses, whose clinical spectrum includes diarrhea, hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) (Karmali, 2003). Infections caused by STEC are considered a public health problem in both developed and developing countries because of the severity of the disease they cause, and the global nature of the food supply chain (Brando *et al.*, 2008). Sources of food for STEC infections in humans include food of animal origins such as meat (especially ground beef), unpasteurized contaminated milk and other vehicles that have been contaminated with STEC like fresh-pressed apple cider, yogurt, and vegetables such as lettuce and other leafy greens (Karmali, 2003). Waterborne transmission and contact with infected animals are two routes that are becoming increasingly recognized. In addition to large widespread outbreaks in the United States, outbreaks of STEC infection have been documented in at least 14 countries in a variety of settings including households day care centre's, schools, restaurant, nursing homes and prisons (Karmali, 2003). The STEC severe gastroenteritis may cause life-threatening HUS, the most serious complication of STEC infection. Most patients with HUS in developed countries have evidence of exposure to Shiga toxin-producing *E. coli* (O'Brien and Kaper, 1998). Hemolytic uremic syndrome (HUS) is a leading cause of acute

renal failure in children and occurs in about 6% of patients with STEC infection which occurred in Washington DC (Griffin, 1998) up to 40% of patients with HUS develop long-term renal dysfunction (Schering *et al.*, 2008) and about 3-5% of patient die during the acute phase of the disease (Karmali, 2003).

However, the primary virulence factor of STEC is the production of one or more types of Shiga toxin (stx1 and stx2 or both) adherence to the intestinal epithelium and colonization of the gut are also essential components of the disease. These STECs are not typically invasive and restricted to the lumen of the gut, in some circumstance Shiga toxin (stx) produced within the intestinal tract can cross the epithelial border and enter the bloodstream (Brando *et al.*, 2008). Both stx1 and stx2 are capable of crossing epithelial borders via an energy-requiring process, and the toxin that moves across the border retains its biologic intestinal as well as systemic dysfunction (Brando *et al.*, 2008). While the route that the toxin uses to pass across epithelial cell barriers is not well understood, it appears to take a transcellular route. This notion is based on the observation that toxin movement is energy dependent and directional with greater toxin movement in the apical-basolateral direction vice-versa (Acheson *et al.*, 1998). Apart from the main virulence factors of STEC (stx), two additional markers also play a role in the pathogenesis of HC and HUS. An outer membrane protein (intimin) encoded by the *eae* gene and enterohaemolysin encoded with *hly A* gene (Paton and Paton 1998; Karmali *et al.*, 2010). This genetic virulence characteristic is often used in epidemiological studies to correlate between strains from various sources (Askari *et al.*, 2010). Molecular sub-typing techniques used in ten of 19 human incidents in Scotland showed that STEC O157 isolates from cattle and humans cases were indistinguishable (Synge *et al.*, 1994). The STEC isolates from animals have been implicated as the cause of diarrhea and hemorrhagic colitis in humans (Gyles and Fairbrother 2004; Radostits *et al.*, 2007; Islam *et al.*, 2008). In Nigeria, studies conducted at South Western part reported the isolation of *E.coli* O157: H7 and other pathogenic *E. coli* strain from human patients with diarrhea (Olorunshola *et al.*, 2000; Okeke *et al.*, 2003). Similarly, the isolation of non-O157 STEC and some EPEC serotypes was reported from faeces of diarrheic calves collected from various farms in Zaria North Central Nigeria (Tekdek *et al.*, 1995). Also, in north-eastern Nigeria, Moses *et al.* (2005) isolated STEC O157: H7 from human and cattle faeces.

Recently, Rahimi *et al.* (2010) reported the prevalence of STEC O157: H7 in animals during processing in Iran. However, STEC is becoming the causes of severe public health concern in the world and associated with food-borne outbreaks leading to the life-threatening disease. The prevalence of STEC serotypes O157, O26, O91, O103, O111, O128 and O145 which associated with public health risk has not been reported in chickens in Maiduguri. It is against this background that this study was conducted to isolate and characterize Shiga toxin producing *E. coli* (STEC) from chickens in Maiduguri, Nigeria.

II. MATERIALS AND METHODS

A. Study Area

This study was carried out in Maiduguri the capital of Borno State, and a commercial nerve centre of the North-eastern region of Nigeria. Maiduguri located in the semi-arid zone of Borno state with an area of 69,436 km² and lies between latitude 10-13°N and longitude 12-15°E. It lies within the Savannah and Sahel vegetation and receives little rainfall. The area falls within the tropical continental north, with a dry season of between four to seven months (November to May), followed by a short wet season from early June to late October (Gisilambe, 1990). Borno State shares boundary with Chad to the northeast, Cameroon to the east and Adamawa state to the south west. According to the census, the population is estimated to be 4,558,668 and ranked 12th out of the 36 state in the country (Gisilambe, 1990).

B. Collection of Samples

A total of three hundred (300) faecal samples were randomly collected from chickens brought to the Maiduguri Monday Market Chicken Slaughter house. One hundred and fifty (150) each of the broiler chickens and village chickens were sampled. All samples were collected in sterile well labeled universal containers for further analysis.

C. Antimicrobial Susceptibility Test

Isolates containing Shiga toxin-producing sero groups (types) tested for antimicrobial susceptibility. The media used for the test include sterile saline solution (0.85%), Mueller-Hinton agar (CM337, Oxoid), and nutrient agar (CM3Oxoid). Antimicrobial Disks (Ampicillin 10µg/disk, Tetracycline 30µg/disk, and Ceftriaxone 10µg/disk; Oxoid UK) were also used for the susceptibility testing, based on the manufacturer's specification and the standard procedures. Prior to preparing the inocula, the agar plates containing the test organism and the control strain were examined, and where culture appears mixed, a fresh sub-culture was prepared. Using a sterile loop, at least 4 to 5 of the isolated colonies were transferred to the tube of the saline. The inocula were emulsified inside the tube to avoid clumping of the cells. The inocula were adjusted to 0.5 McFarland standards by comparing turbidity with the one contained in 0.5 McFarland standards using paper with black lines and adjusted accordingly to give similar turbidity to the standard (McFarland 0.5 equals approximately 10⁸ CFU/ml) (Hendriksen, 2003). The Mueller Hinton agar plates (Oxoid-prepared according to manufacturer's instructions) prior to use, were visually examined to ensure that the plates were free of visible contamination before being dried. Sterile cotton swabs were used to streak the inocula evenly on the surface of the agar. The swabs were rotated several times and pressed firmly inside the wall of the tube, above the fluid level to remove excess inocula from the swabs. Any excess moisture on the agar surface was allowed to be absorbed prior

to application of the antimicrobial disks (Hendriksen, 2003). Sterile forceps were used to fix the disk on the surface of the agar. Finally, the plates were incubated at 37°C for 24-48 hours, the results obtained by measuring zones of inhibition using a meter rule. The results were analyzed and interpreted based on the standards established by Clinical and Laboratory Standard Institute (CLSI, 2014).

D. Statistical Analysis

The data generated from this research were analyzed using descriptive statistics and ANOVA with Turkey Kramer (HSD) test using JMP version 11 (SAS Institute Inc, Cary NC). The analysis was considered significant at $p < 0.05$.

III. RESULTS

Total number 300 faecal samples were obtained and analyses for O157 STEC and non-O157 STEC, using latex agglutination for O157 STEC and the polyvalent dry spot sero check for non-O157 STEC. The results of this study showed that the broiler chickens had 19 (12.5%) and 8 (5.3%) positives for both O157 and non-O157 STECs respectively. On the other hand, the village chickens had 26 (17.3%) and 9 (6%) positive for O157 and non-O157 STECs respectively. The remaining 9 (6%) and 12 (8%) of the *E.coli* isolates obtained from broiler and village chickens respectively, cannot be serotyped with the conventional kit (Table I).

TABLE I: STEC AND NON-STECSEROTYPES OF VILLAGE AND BROILER CHICKENS

Sample Type	Serotype	Positive <i>E.coli</i>	Positive <i>E.coli</i> (%)
Broiler chicken (BC)	O157	19	12.7
(n=150)	Non-O157	8	5.3
	Untype	9	6.0
Village chickens (VC)	O157	26	17.3
(n=150)	Non-O157	9	6.0
	Untype	12	8.0
Total (n=300)	All	83	27.7

Antimicrobial susceptibility test of STECs obtained from broiler, and village chickens showed that the O157 STEC from broilers were sensitive CIP 19 (100%), GEN 19 (100%), TET 19 (100%) and resistant to CTX 18 (95%). The non-O157 STECs from broiler were susceptible to CIP 8 (100%), GEN 8 (100%) and were resistant CTX 8 (100%), AMP 6 (75%)

and TET 6 (75%) (Table II). The O157 STECs from village chickens were susceptible to GEN 26 (100%), CIP 22 (84.6%), TET 21 (80.8%) and CRO 19 (73.1%) and resistant to STP 16 (61.5%). The non-O157 STECs from village chickens were susceptible to CIP 9 (100%) and GEN 9 (100%) and resistant to STP 7 (78%) (Table III).

TABLE II: ANTIMICROBIAL SUSCEPTIBILITY OF STECS IN BROILER CHICKEN IN MAIDUGURI

Antimicrobial Agents	Conc. of Drugs (μ g/disk)	Susceptible				Intermediate				Resistant			
		0157VC		NON-0157VC		0157VC		NON-0157VC		0157VC		NON-0157VC	
		No. of Isolates	No. of Isolates (%)	No. of Isolates	No. of Isolates (%)	No. of Isolates	No. of Isolates (%)	No. of Isolates	No. of Isolates (%)	No. of Isolates	No. of Isolates (%)	No. of Isolates	No. of Isolates (%)
CRO	30	15	79.0	3	37.6	3	16	4	50	1	5	1	12.5
CTX	5	0	0.0	0	0.0	1	5.0	0	0	18	95	0	100
AMP	10	3	16.0	0	0.0	9	47	2	25	7	37	6	75
CIP	10	19	100	8	100	0	0.0	0	0	0	0	0	0
TMP	5	0	0.0	0	0.0	18	95	8	95	1	5	0	0
STP	25	0	0.0	0	0.0	10	53	6	75	9	47	2	25
GN	30	19	100	8	100	0	0.0	0	0	0	0	0	0
NAL	30	9	47.0	0	0.0	10	53	4	50	0	0	4	50
TE	30	19	100	0	0.0	0	0.0	2	25	0	0	6	75
CHL	30	19	100	4	50	0	0.0	4	50	0	0	0	0

CRO = Ceftriaxone, AMP = Ampicillin, CIP = Ciproflaxacin, TMP = Trimethoprim, STP = Streptomycin, GEN = Gentamycin, NAL = Nalidixic Acid, CTX = Cefodizime, TE = Tetracycline and CHL = Chloramphenicol.

TABLE III: ANTIMICROBIAL SUSCEPTIBILITY OF STECS IN VILLAGE CHICKEN IN MAIDUGURI

Antimicrobial Agents	Conc. of Drugs (µg/disk)	Susceptible				Intermediate				Resistant			
		0157VC		NON-0157VC		0157VC		NON-0157VC		0157VC		NON-0157VC	
		No. of Isolates	No. of Isolates (%)	No. of Isolates	No. of Isolates (%)	No. of Isolates	No. of Isolates (%)	No. of Isolates	No. of Isolates (%)	No. of Isolates	No. of Isolates (%)	No. of Isolates	No. of Isolates (%)
CRO	30	19	73.1	5	66.6	3	11.5	3	33.33	4	15.4	1	11.1
CTX	5	1	3.8	0	0	20	76.9	2	22	5	19.2	7	78
AMP	10	10	38.6	0	0	8	30.7	0	0	8	30.7	9	100
CIP	10	22	84.6	9	100	4	15.4	0	0	0	0	0	0
TMP	5	4	15.4	1	11.1	17	65.4	5	56	5	19.2	3	33.3
STP	25	0	0	0	0	10	65.4	2	22	5	19.2	7	78
GN	30	26	100	9	100	0	0	0	0	0	0	0	0
NAL	30	16	61.8	0	0	10	38.5	6	67	0	0	3	33
TE	30	21	80.8	0	0	5	19.2	5	56	0	0	4	44
CHL	30	16	61.5	2	22	10	38.5	7	78	0	0	0	0

CRO = Ceftriaxone, AMP = Ampicillin, CIP = Ciproflaxacin, TMP = Trimethoprim, STP = Streptomycin, GEN = Gentamycin, NAL = Nalidixic Acid, CTX = Cefodizime, TE =Tetracycline and CHL = Chloramphenicol.

IV. DISCUSSION

E. coli is used as an indicator of faecal contamination of water bodies, vegetables, milk, and meat products. The ingestion of the *E. coli* contaminated foods can lead to detrimental health hazards. In this study, high percentage prevalence of O157 STEC serotypes was found among the *E. coli* isolated from broiler and village chickens with a higher distribution of O157 STEC in village chickens. These findings disagree with the earlier reports by Blanco *et al.* (1998), Raji *et al.* (2007) and Geidam *et al.* (2012) who reported that there is an apparent paucity of pathogenic *E. coli* in poultry environment. The inability to type the remaining *E. coli* isolates appear to agree with the earlier reports by Blanco *et al.* (1998), Raji *et al.* (2007) and Geidam *et al.* (2012) which stated that many *E. coli* strains could not be typed. However, there was low prevalence of Non-O157 STEC serotypes isolated in broiler and village chickens compared to the O157 STECs in this study. The non-O157 serotypes isolated in this study are similar to those isolated in faeces of Camels, Cattles and humans in Maiduguri (Mosses *et al.*, 2005; Sakuma, 2014). The finding of the present study showed that the Non-O157 STEC isolates were more resistant to the antimicrobial agents than the O157 STECs. The finding agrees with the earlier reports by Farina *et al.*, (1996) and Galland *et al.*, (2001) on antibiotic resistance especially among Non-O157 STEC. The present studies showed the isolate were sensitive to Ceftriaxone, Ciprofloxacin, and Gentamicin. However, Gentamicin in the present study contradicts the findings of Daini and Adesemowo (2008) on all the STEC that were resistant to Gentamicin. In Nigeria, gentamicin is marketed as an injectable solution and is not commonly used for mass medication in poultry.

This could be the reason for the high susceptibility of STEC isolates to the drug in the study area. The isolates showed to be resistant to Tetracycline and Ampicillin (Non-O157 Village and Non-O157 Broiler chickens), Nalidixic acid, Cefodizime and Streptomycin which is in accordance with the investigations carried out on Tetracycline by Daini and Adesemowo (2008). The indiscriminate use of Tetracycline by poultry farmers and its consequent poor antimicrobial activity could be responsible for the resistant on the STEC isolates in the study area. The findings of this study also showed intermediary activities of Cefodizime and Trimethoprim, Chloramphenicol, on the STEC isolates. This could be probably due to the increased use of antibiotics as feed additives for growth promotion and prevention of diseases in the study area.

Investigation on STEC serotypes O157 and Non-O157 (O26, O91, O103, O111, O128 and O145) STEC showed out of the 300 faecal samples collected at random from broiler and village chickens in Maiduguri, there was high prevalence of Shiga toxin-producing *E. coli* serotypes which has relatively higher prevalence of STEC in village chickens compared with the STEC obtained from broiler chickens.

Ruminants have been identified as the major reservoir of *E. coli* O157 and also appear to be a reservoir Non-O157 STEC (Wieler, 2003) although Non-O157 STEC has been detected in non- ruminant animals (Makino *et al.*, 2000). STEC has been isolated from a variety of domestic animals. However, it is believed that in many cases they are present as transient bacteria that the animals acquired from feeds or water probably contaminated faecal materials from ruminants. This may be the possible reason why STEC was isolated more in village chickens which are free-range domestic animals than in the broilers.

V. CONCLUSION

The findings of this study revealed that Shiga toxin-producing *E. coli* sero groups are found in the faecal samples of broiler and village chickens in Maiduguri with a relatively high prevalence of the O157 STEC strains in Village Chickens. The isolates were susceptible to ciprofloxacin, gentamicin, and Ceftriaxone and were resistant to tetracycline, Ampicillin and streptomycin respectively. This study is the first to report the isolation of O157 and Non-O157 STEC serotypes in chickens in Maiduguri metropolis, Northeastern, Nigeria.

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