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ISOLATION, IDENTIFICATION AND *ANTIMICROBIAL* SUSCEPTIBILITY OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* IN CAT FISH (*clarias gariepinus*) OBTAINED FROM ALAU RIVER AND A COMMERCIAL FISH POND IN MAIDUGURI, NIGERIA

Falmata Ali Abadam[1], Maryam Asheik Jarma[2], Yagana Shettima[3], and *Fusam Shettima kuburi*[4]

[1&2] Department of Food Science Technology, Ramat Polytechnic, P.M.B.1070, Borno State.
[3&4]Department of Science Laboratory Technology, Ramat Polytechnic, Maiduguri, Borno State

Abstract: *This study was carried out to isolate, identify and determine the antimicrobial susceptibility of shiga toxin-producing Escherichia coli (STEC) in Catfish (clarias greipinus). Forty (40) fishes were used for the study, twenty (20) were obtained from Alau River and the other twenty (20) were obtained from commercial (artificial) fish ponds. Swaps were collected from the gill, skin and gut of the fishes. A total number of one hundred and twenty (120) samples of various anatomical parts (gills, skins and guts) were subjected to bacteriological and biochemical examinations for identification and confirmation of E. coli STEC isolate Positive E.coli were sero typed using latex agglutination test for 0157 STEC and dry spot sero check for Non 0157 STEC. The result revealed high isolation of Non 0157 in both Alau River and the commercial fish ponds with isolation of STEC Non O157strains in the commercial fish ponds. Antimicrobial susceptibility revealed all the STEC isolates were susceptible to the antimicrobial agent with high susceptibility to ciprofloxacin.*

Keywords: *Antimicrobial Susceptibility, Catfish, Fish Pond.*

INTRODUCTION

Fish and fish products are the most important source of protein and it is estimated that more than 30% of fish for human consumption comes from aquaculture (Hanstein *et al.,*2006; Yagoub, 2009; Adebayo *et al.,*2012). Fishery products are important not only for nutritional point of view, but also as an item of international trade and foreign exchange earner for a number countries in the world (Yagoub 2009; Adebayo *et al*., 2012). Fish and shell fish are highly perishable and prone to vast variation in quality due to difference in species, environment, habitant and feeding habits (Yagoub 2009; Adebayo *et al*., 2012) . In addition, they can function as carriers of microbial and other health hazards (Yagoub 2009; Adebayo *et al*, 2012). Aquatic bacteria that infect fish belong to three groups: the Gram- negative bacteria (most common) Gram- positive bacteria and the acid-fast bacteria which are obtained from food or from the environment. Gram-negative bacteria cause most of the diseases in tropical fish.

Escherichia coli (*E. coli*) in fish are considered as an indicator of potential sewage pollution. Levels of *E. coli* are used to determine whether local beaches should be posted with "no water contact" advisories. *E. coli* is a bacterium that commonly lives in the intestine of many people, animals and fish (Hanson *et al.,* 2008). There are many strains (types) of *E. coli* that are normal inhabitants in the small intestine and colon and are non-pathogenic, meaning they do not cause disease in the intestine. Nevertheless, these non-pathogenic *E. coli* can cause disease if they spread outside the intestine. The pathogenic strains of *E. coli* may cause diarrhea by producing and releasing toxins (called enterohaemorrhagic *E. coli* or Shiga toxin producing *Escherichia coli*) and cause of food poisoning in fish (Lee and Marks, 2009).
There are many pathogenic strains causing variety of illness in man and animal with associated clinical features and virulence factors depending on the sero groups from a food safety perspective (Askari *et al.,* 2010). These illness ranges from gastrointestinal disturbances like watery diarrhea to more complicated sequelae like the Hemorrhagic Colitis (HC) or Hemolytic Uremic Syndrome (HUS) Dirrheagenic *E. coli* are now classified into six groups: Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E.coli* (EAEC), Diffusely Adherent *E. coli* (DAEC) and Enteroheamorrhagic *E. coli* (EHEC) or Shiga toxin-producing *E. coli* (STEC) (Paton and Paton, 1998; and Evans *et al.,* 2007).

Shiga toxin (Stx)-producing *Escherichia coli* (STEC), also termed Verocytoxin (VT) Producing *Escherichia coli* (VTEC), are the most important groups of Dirrheagenic *E. coli* that is associated with food-borne outbreaks leading to life threatening complications (Paton and Paton, 1998; Beutin *et al.,* 2007). STEC infection can cause gastroenteritis that may be complicated with hemorrhagic Colitis or Hemolytic Uremic Syndrome which is the main cause of acute renal failure in children. STEC strains causing human infection belong to a large, sill increasing number of O, and O: H serotypes. There are over two hundred STEC serotypes identified but many are not implicated in human illness (Bonnet *et al.,* 1998; Paton and Paton, 1998; Karmali *et al.,* 2010). A restricted range of serotypes (O157, followed by O26, O103, O91, O45 and O111) are associated with public health risks, and these serotypes are most frequently isolated from food or animals (Willslaw *et al.,* 2001; Bennett and Bettelheim, 2002). However, other serotypes are becoming a cause of serious public health concern, as STEC isolate from animals
have been implicated as cause of diarrhea and hemorrhagic colitis in humans (Gyles and Fairbrother 2004, Radostits *et al.,* 2007, Islam *et al.,* 2008) . This study is conducted to isolate and characterize shiga toxin producing *E. coli* (STEC) isolated from anatomical parts of *Clarias gariepinus* (Catfish) in Maiduguri Nigeria.

MATERIALS AND METHOD

Sample Collection

Forty (40) live catfish (Africa *Clarias gariepinus)* were collected randomly from Alau River (wild) and a commercial fish farm. The fish were collected in separate sterile polythene bags directly from the fishing net and were transported to the Veterinary teaching and research lab University of Maiduguri.

Sample preparation

Swabs were collected from skin, gill and gut of the 40 fishes obtained from both Alau and the commercial fish pond. The fishes are table fish (Adult), with an average 154.8grm. The samples were named as follows:

AG-for swab samples collected from gills of fish obtained from Alau River.
AS-for swab samples collected from skins of fish obtained from Alau River.
AGT-for swab samples collected from guts of fish obtained from Alau River.
BG-for samples of gills collected from commercial fish pond.
BS-for samples collected from skins of fish obtained from commercial fish pond.
BGT-for samples of guts collected from Alau River respectively.
Culture was done within 2 hours of collection of samples. All swabs were then incubated on MacConkey agar for 24hrs and observed for bacterial growth (Lactose fermenting).

Statistical Analysis

Anova with Tukey Kramer (HSD) test, discriminant test of canonical plotand graph builder were employed in analyzing the data obtained using JMP version 11 (SAS Institute Inc., Cary NC). Analyses were considered significant at $p<0.05$.

Results

Growth on MacConkey Agar Plate

Figure 1 showed the growth of bacteria on MacConkey agar samples of cat fish collected from Alau and commercial fish ponds. Out of the 120 samples, 88(73.32) The remaining 88 samples showed pinkish colony (Lactose fermenting) on MacConkey Agar (18 (90%) AG, 8 (40%) AGT, 10 (50%) AS, 20 (100%) BG, 12 (60%) BGT and 20 (100%) BGS respectively). The Chi-square test showed a value of 36.136 with a significant level of ($p<0.0001$) and contingency coefficient of 0.481. The chi-squared showed the strength of association between the location and the status (presence and absence of growth) which is indicated by pinkish colony i.e. lactose fermenting colony. The contingency coefficient also indicates the length of association between the location and status (0 indicates no association while 1 corresponds to perfect association between the variables.

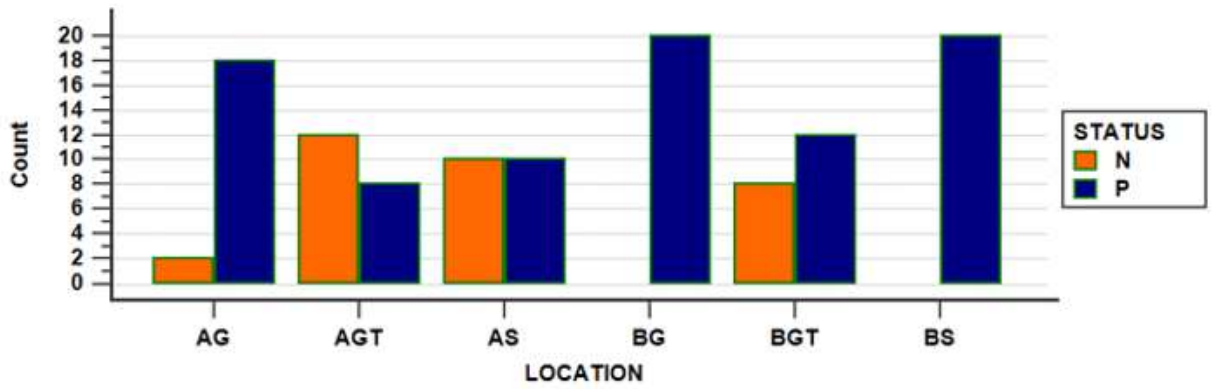

Figure 14: The growth of Bacteria on MacConkey Agar from the gills, skins and guts of Cat fish obtained from Alau River and Commercial fish Ponds

Growth on Eosin methylene Blue Agar Plate

The 88 positive samples obtained from MacConkey agar plate were sub cultured on Eosin Methylene Blue Agar. Fifty two samples gave a greenish metallic sheen which confirms to be *E. coli*, of which AG revealed 12 (23%), AGT 2(3.8%), AS 6(11.5%), BG 10 (19.2%), BGT 6 (11.5%) and BS 16 (30.8%) respectively.

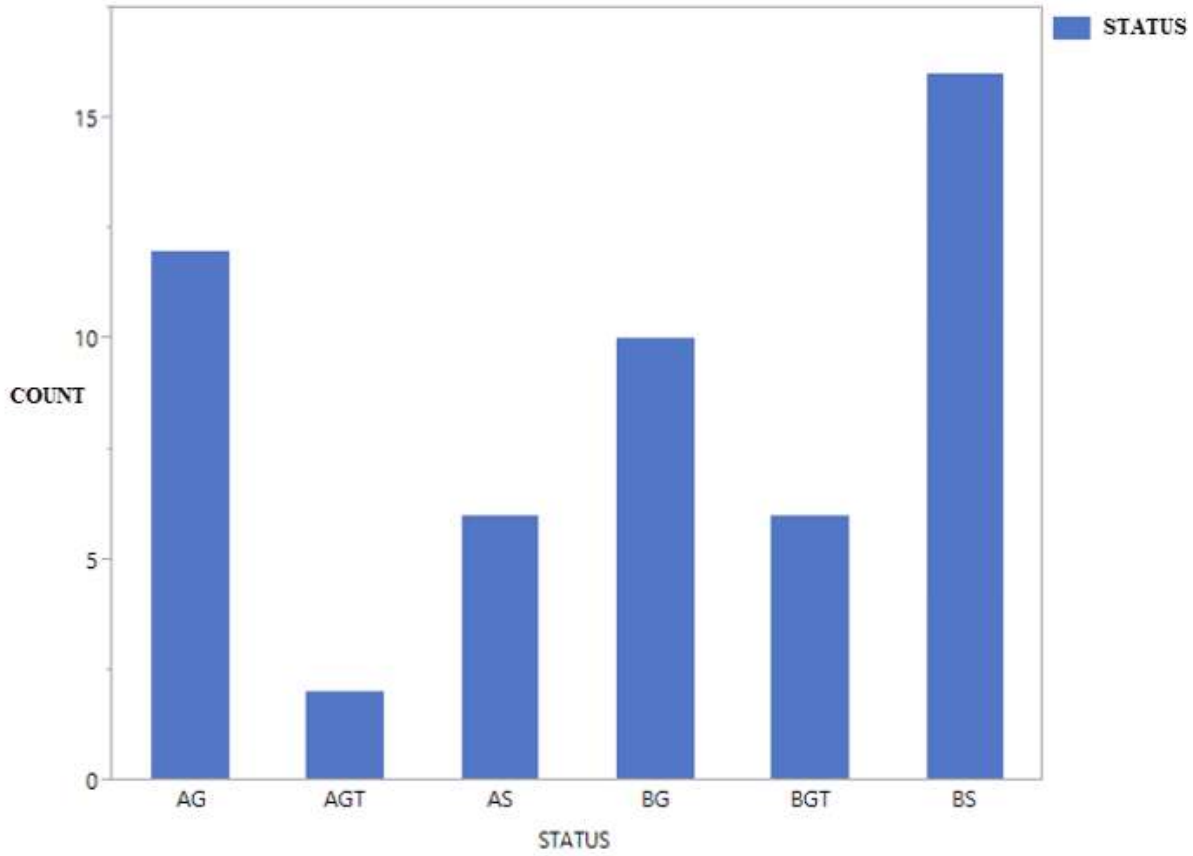

Figure 15: Growth of Culture on Eosin Methylene Blue Agar from the gills, skins and guts of Cat fish obtained from Alau River and Commercial fish Ponds

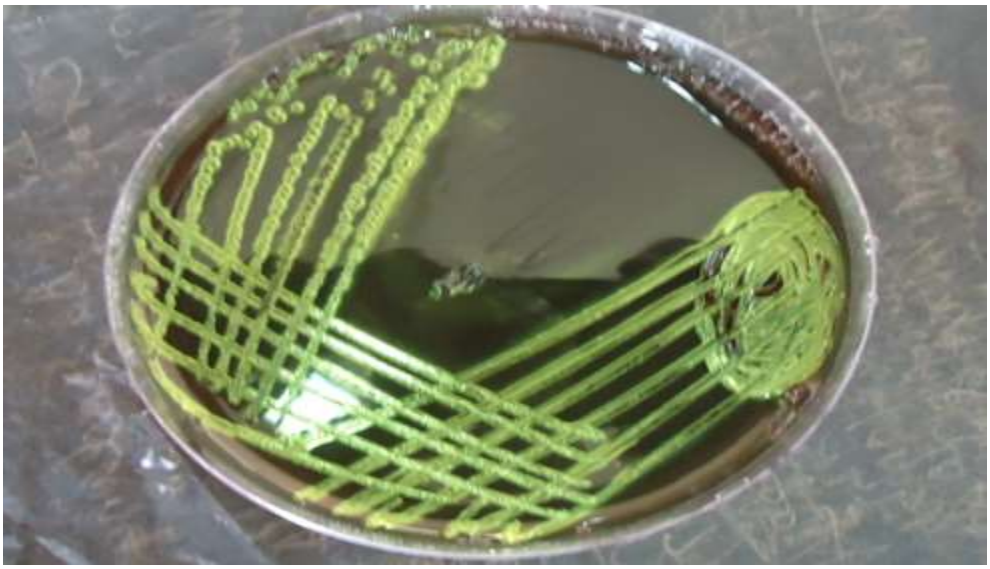

Plate 1: Greenish Metallic Sheen on EMBA

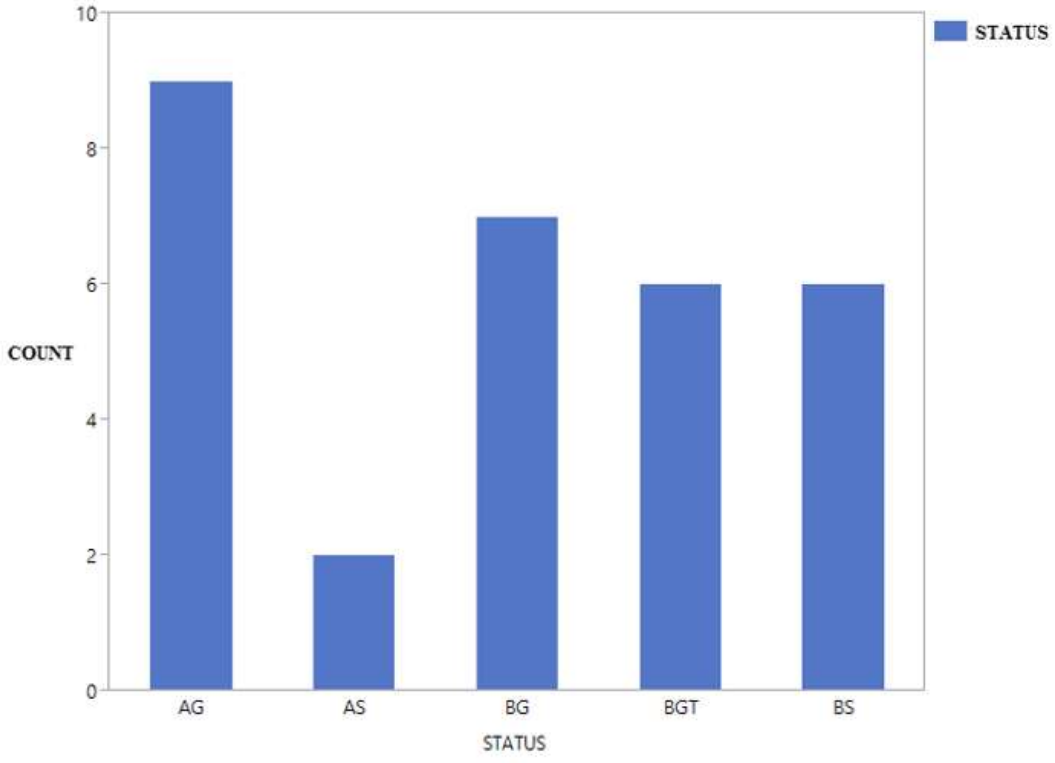

Figure 16: Non 0157 STEC Serotypes isolated from the gills, skins and guts of Cat fish obtained from Alau River and Commercials fish Ponds

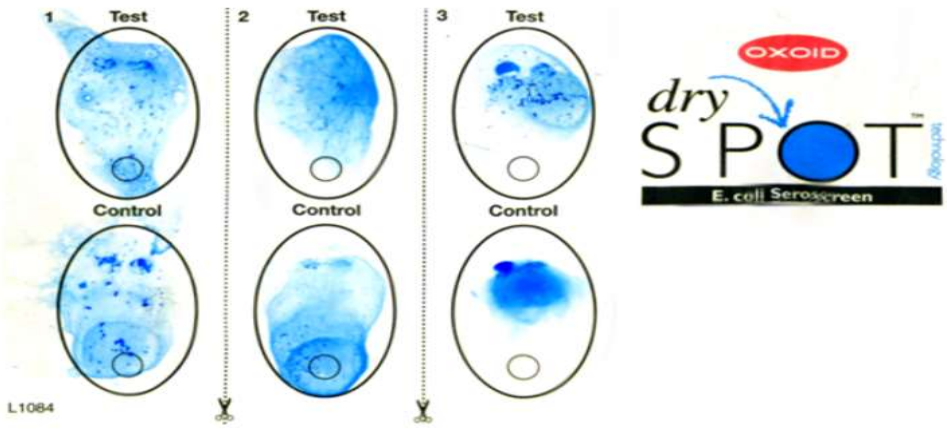

Plate 2: Dry Spot Sero Checks for Non 0157 STEC

Serotyping of *E.coli*
Identification of Non O157 STEC

Figure 3: showed non 0157 STEC serotype isolated from gills, skins and guts of cat fish obtained from Alau River and commercial ponds. Thirty (30) of the samples divulge the presence of non0157 STEC serotype. Out of which 9(30%) from AG, 2(6.7%) from AS, 7(23.3%) from BG, 6(20%) BG and 6(20%) from AS respectively.

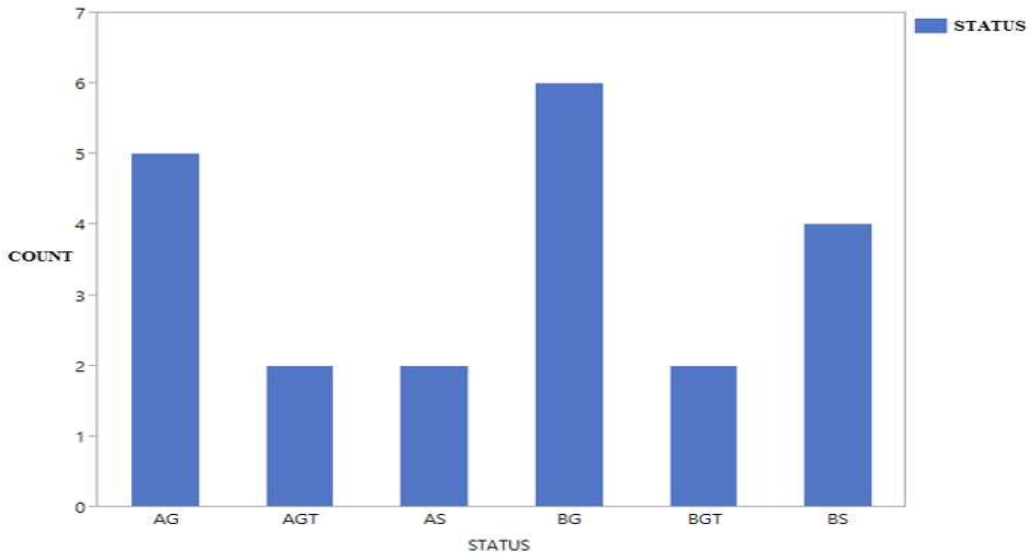

Figure 17: 0157 STEC Serotypes isolated from gills, skins and guts of Cat fish obtained from Alau River and Commercial fish Ponds

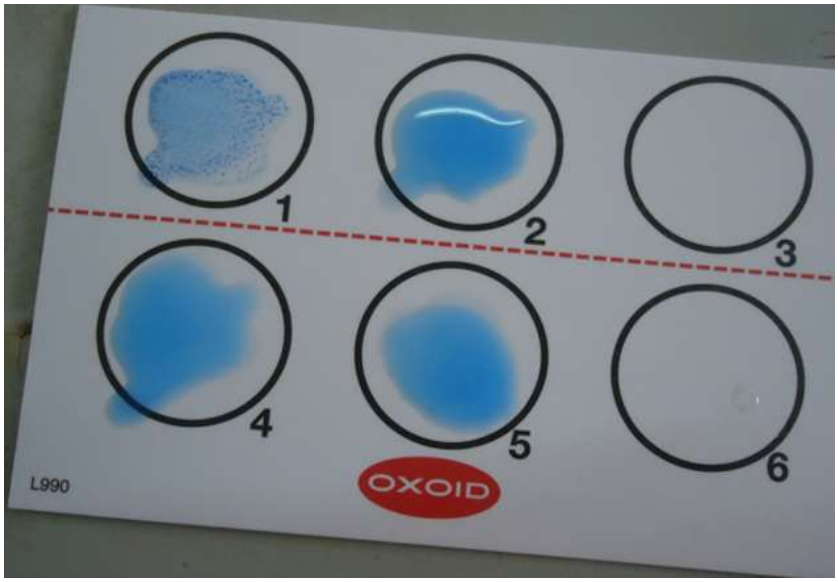

Plate 3: Latex Agglutination test for 0157 STEC Serotype

Identification of O157 STEC

Figure 4: showed 0157 STEC Serotype isolated from gills, skins and guts of cat fish obtained from Alau River and commercial ponds. Twenty one of the samples divulge the presence of 0157 STEC serotype. Out of which; AG 5(23.8%), AGT 2(9.5%), AS 2(9.5%) BG 6(28.6%) BGT 2 (9.5%) and BS 4 (19.0%) respectively.

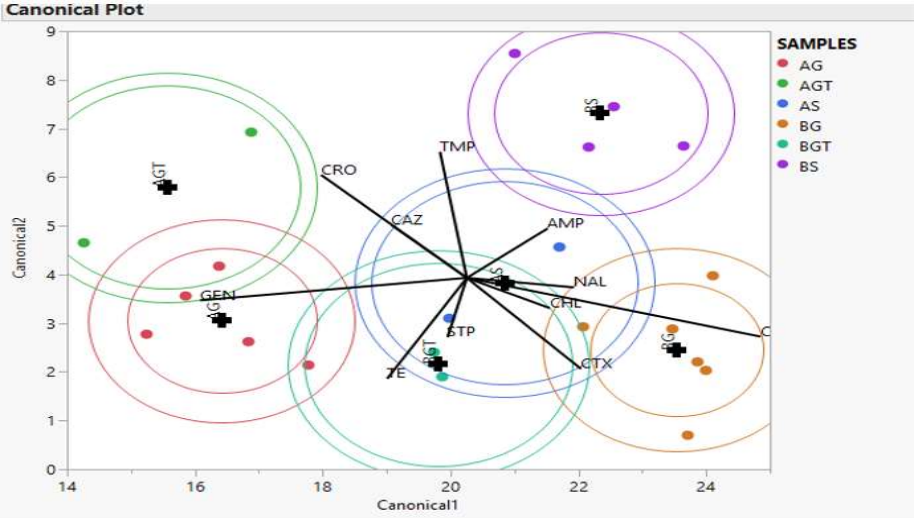

Figure 18: Canonical Plot representation of the susceptibility of different antimicrobials on the 0157 strain of STEC isolated from the gills, skin and guts of Cat fish obtained from Alau River and Commercial fish Ponds; AG, AS and AGT are gills, skins and guts obtained from Alau River while BG, BGT and BS are gills, guts and skin obtained from Commercial fish Ponds

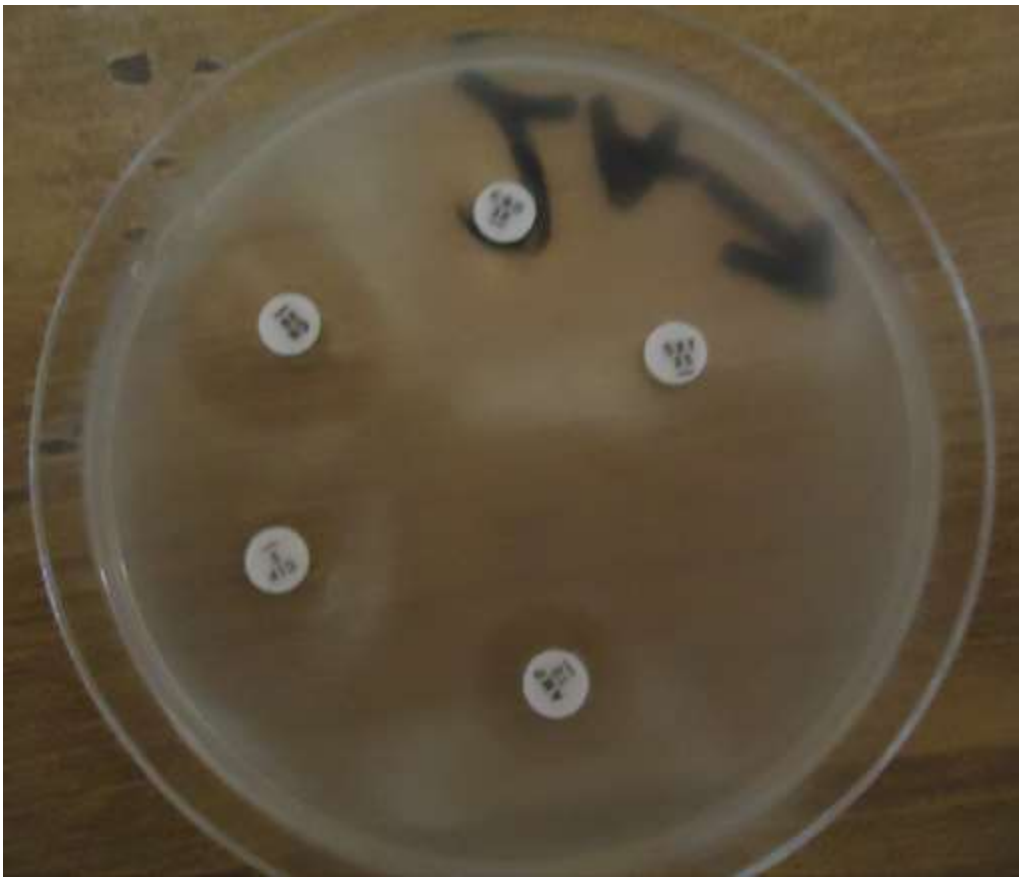

Plate 4: Zone of inhibition of 0157 STEC Serotypes

Antimicrobial susceptibility test

Table 1: Susceptibility of different antimicrobials on the 0157 strains of STEC isolated from the gills, skins and guts of Cat fish obtained from Alau River and Commercial fish Ponds

ANTIMICROBIALS	AG	AS	AGT	BG	BS	BGT
CRO	$18.60^{a} \pm$ 0..78	$18.50^{a} \pm$ 1.17	$20.00^{a} \pm$ 1.17	$19.17^{a} \pm$ 0.68	$21.0^{a} \pm$ 0.83	$18.50^{a} \pm$ 1.17
AMP	$11.80^{a} \pm$ 0.55	$13.50^{a} \pm$ 0.87	$11.50^{a} \pm$ 0.87	$11.50^{a} \pm$ 0.50	$11.50^{a} \pm$ 0.62	$11.50^{a} \pm$ 0.87
CIP	$25.20^{b} \pm$ 0.78	$29.50^{ab} \pm$ 1.24	$26.50^{ab} \pm$ 1.24	$28.50^{ab} \pm$ 0.71	$30.00^{a} \pm$ 0.87	$25.50^{ab} \pm$ 1.24
TMP	$11.20^{a} \pm$ 1.02	$12.00^{a} \pm$ 1.61	$10.00^{a} \pm$ 1.61	$11.50^{a} \pm$ 0.93	$10.75^{a} \pm$ 1.14	$5.50^{a} \pm$ 1.61
STP	$5.00^{a} \pm$ 1.95	$4.50^{a} \pm$ 3.08	$0.00^{a} \pm 0.00$	$6.00^{a} \pm$ 1.78	$2.00^{a} \pm$ 1.18	$0.00^{a} \pm$ 0.00
GEN	$22.00^{a} \pm$ 0.89	$25.50^{a} \pm$ 1.41	$22.00^{a} \pm$ 1.41	$23.33^{a} \pm$ 0.81	$24.00^{a} \pm$ 0.99	$22.00^{a} \pm$ 1.41
NAL	$11.80^{a} \pm$ 0.91	$16.00^{a} \pm$ 1.44	$12.00^{a} \pm$ 1.44	$14.17^{a} \pm$ 0.83	$13.25^{a} \pm$ 1.02	$14.00^{a} \pm$ 1.44
TE	$9.00^{a} \pm$ 1.38	$9.00^{a} \pm$ 2.18	$8.00^{ab} \pm$ 2.18	$4.17^{ab} \pm$ 1.26	$0.00^{b} \pm$ 0.00	$4.00^{ab} \pm$ 2.18
CHL	$12.40^{a} \pm$ 0.69	$13.50^{a} \pm$ 1.08	$10.50^{a} \pm$ 1.08	$13.00^{a} \pm$ 0.63	$12.50^{a} \pm$ 0.77	$13.00^{a} \pm$ 1.08
CTX	$13.60^{a} \pm$ 0.64	$12.00^{a} \pm$ 1.02	$12.50^{a} \pm$ 1.02	$14.67^{a} \pm$ 0.59	$13.00^{a} \pm$ 0.72	$12.00^{a} \pm$ 1.02
CAZ	$14.50^{a} \pm$ 0.59	$16.50^{a} \pm$ 0.93	$15.50^{a} \pm$ 0.93	$14.50^{a} \pm$ 0.54	$15.25^{a} \pm$ 0.66	$14.00^{a} \pm$ 0.93

All values are means ± SE. $^{a, b, ab,}$ within each row, means with different superscripts are significantly different at $P < .05$. CRO = Ceftriazone, CAZ = Ceftazidine, AMP = Ampicillin, CIP = Ciproflaxacin, TMP = Trimethoprim, STP = Streptomycin, GEN = Gentamycin, NAL = Nalidixic Acid, CTX = Cefoxonzime, TE =Tetracycline and CHL = Chloramphenicol; AG, AS and AGT are gills, skin and gut obtained from Alau River while BG, BS and BGT are gills, skin and gut obtained from commercial farms.

Figure 5: showed the canonical representation of the susceptibility of different antimicrobial on the 0157 strain o STEC isolated from the gills, skins, and guts of cat fish obtained from Alau River and commercial ponds.

The different coloured circles in the canonical plots or otherwise named plot represent the location and the types of tissues sampled. Each location and sample are represented by inner and outer circles. These circles are the 95% confidence intervals.

The rays emanate from a point of grand mean and spread in the direction of significant loading or association. Therefore, each antimicrobial agents in the canonical plot migrate to a location that it has effective susceptibility. All the locations and samples have positive and

strong association to canonical 1 and 2. This means there is positive and strong canonical correlations among the canonical varieties.

Trimethoprim (TUP) is susceptible to skin tissue obtained from commercial ponds while Ceftazidine (CAZ) ampicillin (AMP) Ivalidixacid (IVD), Chloramphenicol (CHC), Cefoxonzime (CTX) Streptomycin (STP) are most susceptible to skin tissues obtained from Alau and gills from commercial fish ponds. Gentamycin (GEN) is effective for gills and guts from Alau. Ceftriazone (CRO) is susceptible to guts obtained from Alau. Tetracycline is very effective and susceptible to guts from commercial fish ponds. While Ciproflaxacin (CIP) is most susceptible to gills from commercial ponds.

Table 1 showed the mean inhibition zones exerted by the various antimicrobial agents, hence their susceptibility agents 0157 strains of STEC. All the antimicrobial agents have some degree of ability in inhibiting 0157 strains in all the tissues from both locations excepts tetracycline which did not inhibit 0157 strain on the skin of cat fish from commercial pond. Streptomycin showed no zone of inhibition in the gut of both cat fish from Alau and commercial fish ponds.

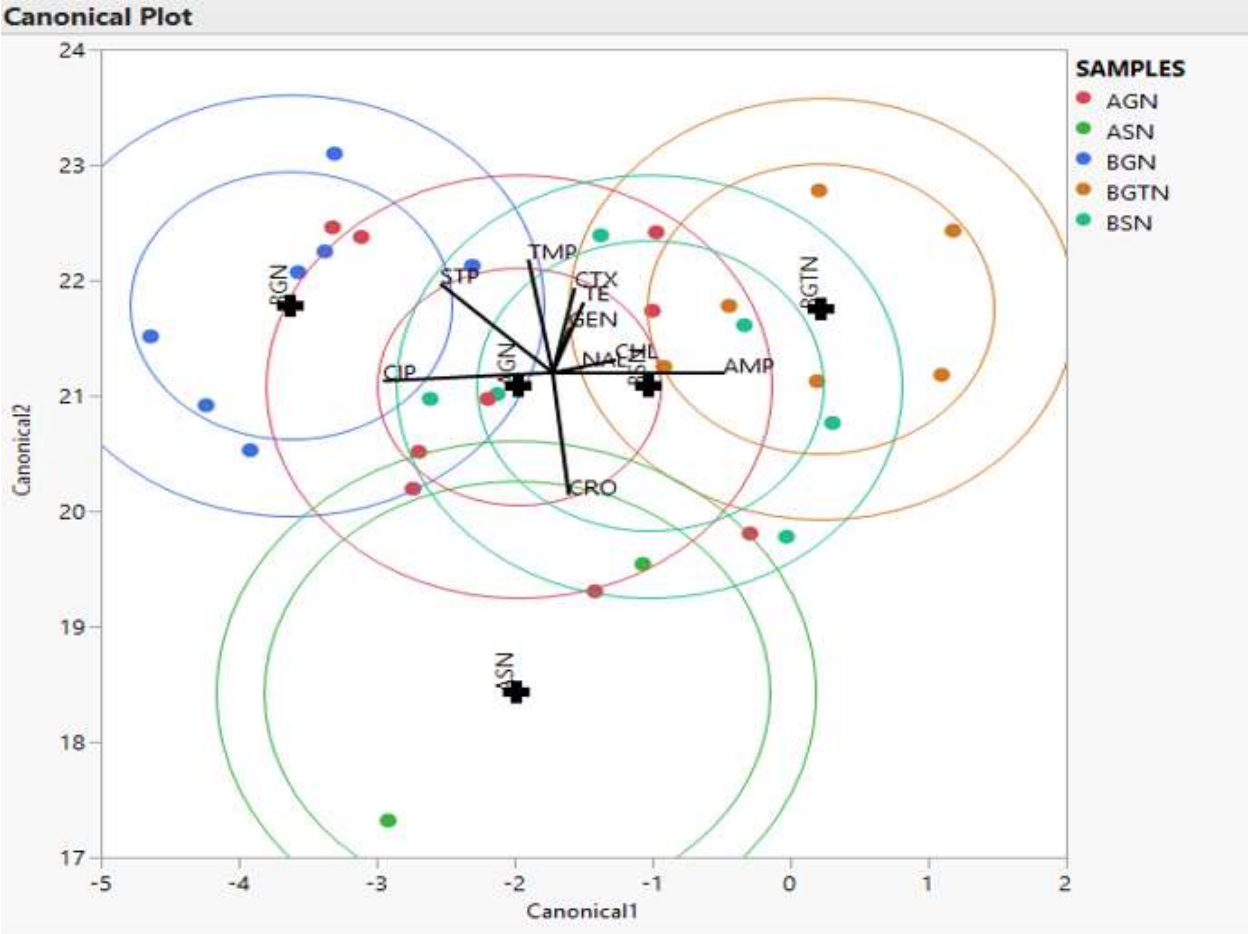

Figure 19: Canonical Plot representation of the susceptibility of different antimicrobials on the Non 0157 strain of STEC isolated from the gills, skins and guts of Cat fish obtained from Alau River and Commercial fish Ponds; AGN and ASN are

gills and skins obtained from Alau River while BGN, BGTN and BSN are gills, guts and skins obtained from Commercial fish Ponds.

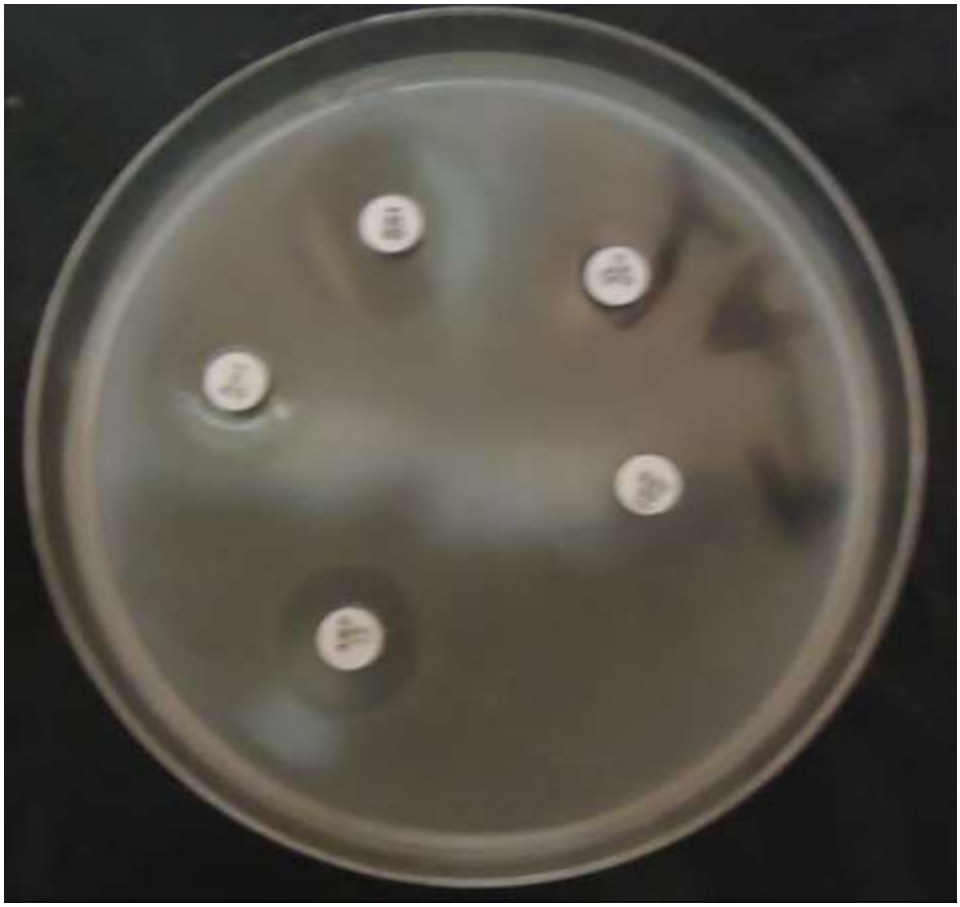

Plate5: Zone of inhibition of Non 0157 STEC Serotypes

Table 2: Susceptibility of different antimicrobials on the Non 0157 strain of STEC isolated from the gills, skins and guts of Cat fish obtained from Alau River and Commercial fish Ponds

Antibiotics	Non-O157 Broiler Chickens (mm)	O157 Broiler Chickens (mm)	Non-O157 Village Chickens (mm)	O157 Village Chickens (mm)
CRO (30µg)	21.50^{a} ± 1.33**(I)**	25.37^{a} ± 0.86**(S)**	23.22^{a} ± 1.26**(S)**	24.58^{a} ± 0.74**(S)**
CTX (5µg)	19.38^{a} ± 0.97**(I)**	18.32^{a} ± 0.63**(I)**	19.33^{a} ± 0.91**(I)**	20.27^{a} ± 0.54**(I)**
AMP (10µg)	11.13^{b} ± 1.03(R)	14.74^{a} ± 0.67**(I)**	11.11^{b} ± 0.97(R)	16.15^{a} ± 0.57(R)
CIP (10µg)	24.13^{b} ± 1.51**(S)**	31.47^{a} ± 0.98**(S)**	28.00^{ab} ± 1.43**(S)**	26.77^{b} ± 0.84**(S)**

TMP (5µg)	$13.25^{a} \pm 0.77$**(I)**	$13.68^{a} \pm 0.49$**(I)**	$11.89^{a} \pm 0.72$**(I)**	$13.04^{a} \pm 0.43$**(I)**
STP (25µg)	$11.00^{ab} \pm 0.50$(R)	$11.58^{a} \pm 0.33$(R)	$9.78^{b} \pm 0.47$(R)	$11.27^{a} \pm 0.28$(R)
GN (30µg)	$21.25^{ab} \pm 0.97$(**S**)	$19.79^{b} \pm 0.63$(**S**)	$22.00^{ab} \pm 0.92$(**S**)	$22.81^{a} \pm 0.54$(**S**)
NAL (30µg)	$12.88^{c} \pm 0.84$(R)	$18.53^{ab} \pm 0.55$(R)	$16.11^{b} \pm 0.79$(R)	$19.04^{a} \pm 0.47$**(I)**
TE (30µg)	$10.63^{b} \pm 0.84$(R)	$17.37^{a} \pm 0.55$(**S**)	$11.44^{b} \pm 0.79$(R)	$17.50^{a} \pm 0.47$(**S**)
CHL (30µg)	$17.25^{ab} \pm 0.79$**(I)**	$19.58^{a} \pm 0.52$(**S**)	$15.89^{b} \pm 0.75$**(I)**	$17.62^{b} \pm 0.44$**(I)**

All values are means ± SE. $^{a, b, ab, c}$, within each row, means with different superscripts are significantly different at $P < .05$. Letters **I**, **R** and **S** indicate the intermediate, resistant and sensitive performance of the antimicrobial agents. Source: (CLSI, 2014); All values are means ± SE. $^{a, b, ab,}$ within each row, means with different superscripts are significantly different at $P < .05$. CRO = Ceftriazone, AMP = Ampicillin, CIP = Ciproflaxacin, TMP = Trimethoprim, STP = Streptomycin, GEN = Gentamycin, NAL = Nalidixic Acid, CTX = Cefoxonzime, TE =Tetracycline and CHL = Chloramphenicol; AGN, ASN and AGTN are gills, skin and gut obtained from Alau River while BGN, BSN and BGTN are gills, skin and gut obtained from commercial farms.

Figure 6: showed canonical representation of the susceptibility of different antimicrobials on the non 0157 strain of STEC isolated from the gills, skin and guts of cat fish obtained from Alau River and commercial fish ponds.

Samples collected from the guts of commercial fish pond that are non 0157 STEC isolates (BGTN) have weak correlation to canonical one variates and a strong correlation to canonical two variates AGN, ASN and BSN have weak negative correlation to canonical one and a strong positive correlation to canonical two variates. BGN has strong negative correlation to canonical one and a strong positive correlation to canonical two variates. These means there is a weak positive correlation and strong positive correlation between the canonical variates of BGTN. That means there are weak negative correlation and strong positive correlation between the canonical variates of AGN, ASN and BSN.

That means there is strong negative correlation and strong positive correlation between canonical variates of BGN. TMP is to non 0157 strain of STEC on STRIA susceptible to BSN (skin) BGN (Gills) and BGTN guts and from commercial ponds and gills of fish from Alau River. Cefoxozime is susceptible to non 0157 of STEC on gills of Alau skin and guts of commercial fish pond. Tetracycline (TE), gentamicin, chloramphenicol, Nalidixic acid and ampicillin have the same susceptibility with Cefoxonzime. Ceftazone is susceptible to gill of non 0157 strain of STEC and skins of Alau fishes obtained from Alau River and skin obtained from commercial farms. Ciproflaxacin and streptomycin are susceptible to non 0157 strains of STEC found on the gills and skins from commercial ponds and gills of fish obtained from Alau River.

Table 2 showed the mean inhibition zone exerted by the various antimicrobial agents. Hence heir susceptibility against non 0157 strains of STEC. All the microbial agents have some degree of ability in inhibiting non 0157 strains in all the tissues from both locations except ampicillin and Ciproflaxacin. Ampicillin is most effective in inhibiting non 0157 strain STEC on the sins and guts of commercial fish ponds whereas ciprofloxacin is most effective in inhibiting non 0157 strains of STEC found in the gills of cat fish obtained from commercial fish ponds.

DISCUSSION

Fish in their natural habitat may not be free from bacterial infestation but the rate is highly dependent some key environmental factors as well as the species of fish and bacteria in question. Certain environmental factors tend to encourage the rate of infestation. *E. coli* is a common disease of fresh water fish especially under cultured condition (Baya and White, 1997) and play an important role in economic losses among fish industry. The present study was carried out to isolate, identify and determined the antimicrobial susceptibility pattern of Shiga toxin producing *E. coli* in anatomical parts of cat fish obtained from Alau River and a commercial fish pond.

From the findings of this study, lactose fermenting organisms producing pinkish coloring on MacConkey Agar was found mostly from gills and skins of cat fish from commercial pond. The result agrees with those of Damba *et al.* (2014) where they reported that fishes reared in fish pond are more prompt to bacterial infections.

The colonies were sub-cultured on EMBA to confirm *E. coli.* The *E.coli* isolated differs from one area to another depending on their location and anatomical parts. Fishes obtained from commercial fish pond had higher isolation of *E. coli* compared to that collected from Alau river. The higher isolate were collected from skins and gills of fish obtained from commercial fish pond. The gills of fish obtained from Alau River had high isolate of *E.coli.* The result agrees with findings of Damba *et al* (2014) which reported high isolate of E.coli in both skins and gills of fish from commercial fish pond.

The result on the serotype showed both Shiga toxin-producing *E.coli* Alau river fish had the highest Non 0157 STEC compared to the other anatomical parts of the Alau River and that of the commercial fish pond. An interesting finding was that there was no Non 0157 STEC in the guts of the fish from Alau River. From the overall findings of Non O157 STEC, the strains are predominantly high in the commercial fish pond as all the anatomical parts had the bacteria infesting on them.

Similar to the findings of the Non 0157 STEC, the O157 were also isolated in both the commercial fish pond and Alau River. However in this case, the gills obtained from the commercial fish pond had high occurrence of the isolate followed by the gills obtained from Alau River.

For the antimicrobial susceptibility testing, using a canonical plot analysis on the antimicrobial pattern we arrived at the specific efficacy of these antimicrobials on the

isolates obtained from Alau River and the commercial fish ponds. The pattern showed that all the O157 STEC were susceptible to all the antimicrobials. These include gentamicin, ceftazidine, nalizilic acid, chloramphenicol, cefoxazime, and tetracycline. While O157STEC obtained from both commercial fish pond and Alau River, were less susceptible to cefriazone and trimethoprim

From the findings, fish from the commercials fish ponds have the higher O157 STEC distribution especially the non O157 STEC however, gills from Alau River had high Non0157 STEC this could be as a result of some certain environmental and biological factors. The findings of the commercial (artificial) fish pond, one may not be out of place to conclude that the presence of STEC on the skins and gills maybe as a result of low number of pond keepers probably because the farm owners will like to minimize cost and maximize profit by keeping staff trend as low as possible. The idea is usually to make optimum output from the few workers which sometimes proof abortive over time. Sometimes, experience maybe a major shortcoming because farm owners also stay away from employing well-trained aquaculture personnel. This may further have its way on the type of feeds being ministered to the fishes. When indiscriminate use of feeds is introduced, the consequence can also be seen on the rate of bacteria infestation. Another reason for prevalence of bacteria in a fish pond could be the irregular draining of the ponds while the water in the river is a free flowing water. The irregular draining of te on could create a situation that provides a favorable condition for bacteria production and development in the host bodies . The hygienic level of a fish pond begins the managers. This is because man is the major cause of problem to his own environment. Therefore, human entry to fish pond must be well screened to ensure the safety of the inhabitant and where possible restrict the number of people that visit the pond because the finding from this study may be probably be as a result of unrestricted entry to the farms by the public. The use of bare hands when feeding the fishes by the farmers is also likely to have contributed to the observed bacteria pathogens and perhaps the use or dirty equipment such as hand nets and cast nets when catching the fishes. Although, the bacteria species found in the fishes in the present study did not cause mortality to the fishes probably because the fishes have strong host defense response yet the specie are both pathogenic and opportunistic which could be involved in causing fish disease (Efuntoye *et al.,* 2012). In addition, *E.coli* could also involve in the transmission of diseases to humans. Fish and fish products have been reported as the vehicles of food-borne bacterial infections in humans (Novotyn *et al*, 2004; Hastein *et al*, 2006; Efuntoye *et al*, 2012). The isolates were isolated from apparently healthy fishes indicating that the organism does not constitute a serious threat to the fishes in the environment of studies. However, these organisms are off public health significance. The *E.coli* recovered was also identified from healthy cat fish in Ago-Iwoye of Oyo state. (Efuntoye *et al*., 2012). Also, a number of investigators have reported the occurrence of pathogenic bacteria amongst which is *E.coli* in freshwater fish aquaculture environment (Ajayi 2012; Emikpe *et al,* 2011; Udeze *et al*, 2012; Adedeji *et al*, 2012). The presences of these bacteria in fish pose a threat to fish consumers as this organism have been implicated in numbers of diseases. *Escherichia coli* strains have been implicated to gastro-enteritis and urinary tract infections (Udeze *et al*, 2012).

Conclusion

Base from the findings from the study, one can conclude Non O157 STEC strains are strongly associated with fishes obtained from the commercial (artificial) ponds especially the skins and gills.

Recommendation

The study only covered the isolation of *E.coli*, STEC in specific. Other pathogenic *E.coli* and bacterial species would be isolated for future studies. Also, the Non O157 STEC strains were obtained using a polyvalent dry spot sero check. A monovalent dry spot sero check would be used for further studies to determine which strain of the Non O157 STEC infests the fishes more.

REFERENCES

Acheson, D, Lincicome L. Jacewicz M, KevischA. (1998). Shiga toxin introduction with intestinal epithelial cells *Escherichia coli* 0157:H7 and other Shiga toxin producing *E.coli* strains. Washington D.C. ASM Press.

Adams M.R, Moss M.O, (2008): Food Microbiology (Third Edition) The Royal Society o chemistry, Cambridge, UK 2008 179pp.

Adebayo. Tayo B.C; Odu N. N, Anyamele L.M, Igwiloh NJPN; Okonko I.O. (2012)

Adesiyun A.A and Kaminjolo J.S. (1992). Susceptibility to antibiotics by *Eschericha coli* strains isolated from diarrhoeic and non –dirrhoeic livestock in Trindad. *Revue Elev Med Vet Pays Trop:* 45:260-262.

Ajayi A.O, (2012): Bacteriological study of cat fish clarias graiepinus, from fish pond source in Akungba-Akoko Community Nigeria British Microbiological Research Journal 2(1):1-9.

Ake, J., Jelacic, S., Ciol, M., Watkins, S., Murray, K., Christie, D., Klein, E., Tarr, P. (2005). Relative NephroprotectionDuring Escherichia coli O157:H7 Infections: Association With Intravenous Volume Expansion. *Pediatrics* **155**(6): 2004-2236.

Akinjogunla, O.J., Inyang, C.U. and Akinjogunla, V.F. (2011). Bacterial species associated with anatomical parts of fresh and smoked Bonga fish (*Ethmalosafimbriota*): Prevelance and susceptibility to Cephalosporins. *Research Journal of Microbiology* 6(1): 87-97.

APHA (American Public Health Association) (1999): Standard Method for Examination of Water and waste water, American water works Association, water Environment Federeation, 9020B.

Askari, M.T. ZahaeiSalehi, M. RabbaniKhorasgani H, Tadjbakhsh, G. NikbakhtBrujeni, M. G. Nandalian (2010) doi: 10.1136/vr.c4009.*Veterinary Record 167:858-861.*

Benefits, Public Health Hazards and Risk Associated with Fish Consumption. New York Science Journal S (a):33-61

Bennett j. and Bettelheim K. A. (2002). Serotypes of non -0157 verocytotoxigenic*Escherichia coli* isolated from meat in new Zealand. Comparative immunology, microbiology and infectionsDiseases 25, 77-84.

Bentley R. and meganathan R. (1982). "Biosynthesis of vitamin K (menaquinone) in bacteria" *microbiol. Rev.* 46(3):241-80.

Beutin, L.A.Miko, G. Krause, K. peris, S. Haby, K. Steege, and N. Albrecht (2007). Identification of human-pathogenic strains of Shiga toxin-producing *Escherichia coli* from food by a combination of serotyping and moleculartyping of Shiga toxin genes. *Appl. Enron. Microbiol.* 73:4769-4775.

Boerlin P. Mc E.wan S., BoerlinPetzold, F., Willson J, Johnson R, Gyles C.(1999). Association between virulence factors of Shiga toxin-producing *Escherichia coli* and Disease in Human.*Journal of Clinical Microbiology*, 37(3) 497-503.

Castellanti A. and charlmers A.J. (1919). Manual of Tropical medicine, 3rd ed., Williams wood and co., New York. Pp 20-30.

Cohen, M.L. (2000). Changing pattems of infectious disease. Nature 406:762-767.

Damton, N.C.,Tumer, L. Rojevsky, S. and Berge, H.C.(2006). "on torque and tumbling in swimming *E. Coli*" *j. Bacteriol.* 189(5):1756-1764.

Danba, E.P., Bichi, A.H., Ishaku, S., Ahmad, M.K., Buba, U., Bingari, M.S. Barau, B.W. and Fedelis U.S. (2014). Occurrence o pathogenic bacteria associated with Clarias gariepinus in selected fish farms of Kumbotso Local Government Area of Kano State, Nigeria. *Bayero Journal of Pure and Applied Sciences* 7(2): 145-149.

Efuntoye, M.O, Olurin, K.B and Jegede, G.C (2012): Bacterial flora from Healthy clarias gariepinus and their Antimicrobial Resistance patter. Advance Journal of Food Science and Technology 4(3):121- 125.

Emikpe, B.O, Adebisi, T. and Adedeji, O.B (2011). Bacterial load on skin and stomach of clarias gariepinus from Ibadan, Southwest Nigeria. Journal of Applied Science Research 7(7):1047-1051.

Evans, Jr, Dole J,Dolares D. Evans (2007) *Escherichia coli*: medical microbiology 4th edition university of Texas medical branch at Galveton. Pp322-323.

Fotadar U., P. Zaretoff and L. Terracio (2005) growth of *E. coli* at elevated temperature. *J. Basic microbiol.* 45 (5): 403-404.

Gay J.M and Hunsakar M.E (1993). Isolation of multiple salmonella serovars from a dairy two years after clinical salmonellosis outbreak. *Journal America veterinary medical association* ; 203: 1314-1320

Griffin P. (1998). Epidemiology of Shiga toxin producing *Escherichia coli* infection inhuman in the United State *Escherichia coli* 0157:H7 and other Shiga toxin-producing *E. coli* strain Washington D.C, ASM Press.

Gyles C.L. and Fairbrother J.M (2004). Escherichia coli. In pathogenesis of bacterial infection in animals. 3rdedn. Eds Gyles C.L., Prescott J.F. ,songerJ.G.andthoen C. O.Black well pp 193-223

Hald, B. Scovgaard H., BangD.D (2004) flies and Campylobacter infectious of broiler flocks Emerging infectious disease: 10:1490-1492.

Hanson, S., Austin, B. and Austin, D. A. (2008). Bacterial fish pathogens. Diseases of farm and wild fish. Springer-Praxis Publishing Ltd. United Kingdom.

Hastein, T.B, Hjeltnes, J., Lillehaug, U., Skare, M., Berntssen, H. and Lundebye, A.K (2006): Food safe safety hazards that occur during the production stage: challenge for fish farming and the fishing industry. Review in Science and technology 25:607-625.

Hemls M. Vastrup P, GermerSimid P, Molbak K. (2002) excess mortality associated with antimicrobial drug resistant S. typhimurium. Emerging infectious diseases. 8 (15):490495.

Hendrisken, R.S. (2003) laboratory protocols level 2 training course susceptibility testing of salmonella using disk diffusion. WHO-Global sam surveillance. Pp 45.

Ingledew W.J. and poole R.K. (1984) the respiratory chain of *Escherichia coli* "*microbiol Rev*. 48 (3):2227-71.

Islam. M.Abba S. mondolEnnedeboer, Rijket. R. Buemer, marcel H. Zwietering, Kaisar A Takulder and Annet E. Hauvelink (2008) prevalence and genetic characteristic of Shiga toxin-producing *E.coli*isolate from slaughtered animals Bangladesh. Journal of App and environ. *Microbiol.* 74:No(17);5415-5421.

Johnson J.R. Murray A.C. Gajewski A. (2003 isolation and molecular characterization of nalidixic acid resistant extraintestinal pathogenic Escherichiacoli from retail chicken products. Antimicrobial agentschemotharaphy; 47:2161-2168.

Karmali M. (2003) the medical significance of Shiga toxin-producing Escherichia Coli infections.In overview *E.coli* Shiga toxin methods and protocol Totowa New jersey Human Press'

Karmali M. A., Gannon V. and Sargeant J.M. (2010) verocytotoxin-producing *Escherichia coli* (VTEC) Veterinary Microbiology 140, 360-370.

Kubitscheck H.E. (1990) cell volume increase in *Escherichia coli* after shift to richer media*. J. bacterial.,* 172(1):94-101.

Lambie N, Ngeleka M, Bron G, Ryan J. (2001). Retospective study on *Eschericha coli infection* in broilers subjected to postmortem examination and antibiotic resistance of isolates in Trindad. *Avian Disease:* 44:155-60.

Lee, M.D. and Marks, M.D. (2009). *E. coli* 0157:H7. Digestive disease Myths Slideshow

Lee, M.D. and Marks, M.D. (2009). Identification and typing of Vibrio anguillarum: A comparism of different methods. Syst. Appl. Microbiol. 18:285-302.

Madigan, M.T. and MartinkoJ.M .(2006) Brock biology of microorganisms (11thed) pearson march SB. And Ratman S. Sorbitol-MacConkey medium for detection of Escherichia coli. 0157:H7 associated with hemorrhagic colitis. *J clin. Microbiol.* 23:869-92.

Martin L. J., Fyfe M, Dore K, Buxton J, A, Pollari F, Henry B, Middleton D, Ahmed R, Jamieson F, Ciebin B, McEwen S.A, and Wilson J.B. (2004). Multi-Provincial Salmonella Typhimurium case-Control study steering committee: Increased burden of illness associated with antimicrobial resistant Salmonella enteica serotype typhimurium infections. *Journal of Infectious Disease*: 189:377-384.

Mathew A. G, Upchurch W, and Chattin S.E. (1998): Incidence of antibiotic resistance of fecal *Eschericha coli* isolated from commercial swine farms. *Journal of Animal Science*, 76:429-434.

Mead p. Slutsker, L. Dietz V, McCaign L, Bresee J. Shapiro C. (1999) food related illness and death in the United State emerging infectious disease. 5:607-25.

Meng J, Doyle MP. 2002. Introduction. Microbiological food safety. Microbes Infect 4:395-7.

O'Brien, .A.D., G.D. LaVeck, M.R. Thompson, and S. B. formal. (1982) production of shigella dysenteriea type 1-like cytotoxin by *Escherichia coli. J. infect. Dis*. 1446:763-769.

Ogden, I.D., M. MacRae, and N.J. C. Strachan. (2004). Is prevalence and shedding of *E.coli* 0157 in beef cattle in Scotland Seasonal? *FEMS microbiol. Lett*. 233-:297-300.

Ojo. O.E. Oyenkule M. A. Ogunleye A.O. Otesile E.B. (2009) E.coli 0157:H7 in food animals in part of S/Western Nigeria: prevalence and invitro antimicrobial susceptibility. *Tropical Veterinarian* 26:23-30.

Olatoye, I.O. (2010).the incidence and antibiotics susceptibility of *Escherichia coli* 0157:H7 from beef in Ibadan Municipal, Nigeria. Afr J. Biotech; 9: 1196-1199.

Paton J.C. and A.W. Paton (1998). Pathogesis of Shiga toxin producing *E.coli* infections. Journal. Clinical microbiol. Rev. 11:450-479.

Philips I, Casewell M. Cox T, De Groot B. Friis C, Jones R, Nightingale C, Preston R, Waddell J. (2004). Does the use of antibiotic in food animal pose a risk to human health? A critical view of published data. *Journal of antimicrobial chemotheraphy* 23:28-52.

Radostis O. M. Gay. C. C. Hinchcliff K. W. and constable P.D. (2007) disease associated with bacteria iii veterinary medicine: A Textbook of the disease of cattle, horse, sheep, pigs, and goats. 10th edn. Saunders. Pp855-856.

Sofos J.N. (2008). Challenges to meet safety in the 21st century. *Meat Science*: 78:3-13.

Tarr, P.I. (1995). *Escherichia coli* 0157:H7 clinical diagnosis, and epidemiological escape of human infections. *Clin. Infect Dis.* 20:1-8.

Umolu P. I. Ohenhen E. R. Okwu I. G. Ogiehor I.S. (2006). Multiple antibiotics resistant index and plasmid of *Escherichia coli* in beef Ekpoma. *Journal of American science*: 2(3):22-28.

Vogt R.L. and Dippold L. (2005). E.coli 0157 outbreak associated with beef. Public health report. 120(2):174-178.

White D.G. Zhaos S. Singh R. McDermott P. F. (2004). Antimicrobial resistance among gram negative food borne bacterial pathogens associated with food of animal origin. Foodborne pathogens and Diseases. 1:137-152.

Willshaw, G.A. Cheasty T., Smith H.R. O'Brien S.J. and Adak G.K. (2001). Verocytoxin-producing *Escherichia coli*. (VTEC). From human infection in England and Wales: 1995- 1998 *Journal of Medical Microbiology* 50:135-142.

World health organization (WHO) (2000). Global principle for the containment of antimicrobial Resistance in animals intended for food. Report of a WHO consultation with the precipitation of food and agricultural organization of the United State Nations and the office international des Epizooties WHO /CDS /CSR /APH /APH / 2000 .4 htt://www.who.int.emc accesed on 21 st December, 2009.

Zhao S. White DG, Ge B, Ayers S, Friedman S, English L, Wagner D, Gaines S, Meng J. (2001) identification and characterization of integronmediated antibiotic resistance among Shiga toxin-producing *Escherichia coli* isolates. *App. Environ. Microbiol:* 67:1558-1559s