Detection and Antibiogram of *Escherichia coli 0157* from Feral and Cultured Tilapia (*Oreochromis niloticus)* in Ibadan, Nigeria

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Abstract: **One of the most important *Escherichia coli* strains known is *E. coli* O157 and is known to cause important human diseases including food poisoning, gastroenteritis, septic shock, etc. Its isolation from food materials and resistance to antibiotics are of increasingglobal-public health concerns. This study investigated prevalence and antibiogram of *E. coli* O157 from *Oreochromis niloticus* (*O. niloticus*)..A total of 156 samples consisting of gills, intestines and skins were collected from 52 *O. niloticus* obtained from Egbeda(A), Ido(B), Ibadan-North East(C) and Ibadan North-West(D) Local Government Areas of Ibadan for bacteriological analysis. *E. coli* 0157 Isolation and identification were performed using the EU, 2017 Reference Laboratory standards forenterobacteracea isolation and characterization, and antibiogram of *E. coli* O157was performed using standard methods.In this study, the overall prevalence of *E. coli* O157 obtained from *O. niloticus* was 62.5% with prevalence of 62.5%, 37.5% and 87.5% from gill, intestine, and skin, respectively, while the overall location prevalence were observed as: 65.4% (A), 65.4% (B), 66.7% (C) and 50.0% (D). Isolates exhibited resistance patterns comprising: 100.0%(Ceftazidime, Cefuroxime and Meropenem), 91.7%(Cefotaxime),83.3%(Tetracycline), 41.5%(Cotrimoxazole),75.0%(Ceftriaxone), 16.7%(Gentamycin), 8.3%(Ciprofloxacin), and 0.0%(Amikacin, Chloramphenicol). Multi-drug resistance patterns were observed as: CRX- CFZ-MEM-(100%), CRX-CTR-CTX-CFZ-MEM-(83.3%), CRX-CTR-CTX-CFZ-MEM-TET(66.7%), CRX-CTR-CTX-CFZ-MEM-TET-COT(58.3%) and CRX-CTR-CTX-CFZ-MEM-TET-COT-GEN(8.3%). Prevalence of *E. coli* O157 in *O. niloticus* obtained from Ibadan andits multi-drug resistance patterns observed in this studyindicate aquaticcontaminations, and abuse of antibiotics, portendingfood safety and public health threats.**

Keywords: **Prevalence, *Escherichia coli 0157*, Antibiogram, and *Oreochromis niloticus.***

I. INTRODUCTION

Aquaculture industry has recently become one of the fastest growing sources of animal protein with global acceptability cutting across all socio-demography and religion sects (Adebayo-Tayo*et al.,* 2010, FAO, 2018). Nigeria, like many other nations of the world, is one of the producers of aquatic products which have favourably competed with meat from other food animals (Adebayo-Adetayo*et al.,* 2010). The acceptance of fish can be attributed toits importance in maintenance of health and wellbeing, distinctively, for unique taste, flavour and good texture(Adebayo-Adetayo *et al.,* 2010). *Oreochromis niloticus* commonly known as Tilapia,belonging to the tilapiine group has become the most cultured and acceptable fish in many parts of the world (José *et al.,* 2017). It has gained worldwide acceptance exemplified by minute amounts of saturated fat, calories, carbohydrates, and sodium, as well as micronutrients, phosphorus, niacin, selenium and potassium. (Robin *et al.,* 2006; Ayanda *et al.,*2018). Despite the enormous health and dietary benefits of tilapia, it is a potential source of food-borne infections to human consumers and/or handlers at every stage of production (Norma *et al.,* 2018). Although, many of the microbes associatedwithfish are informed by theaquaticenvironmentin which they are produced, majority of them are potential spoilers, and potential health threat to human upon consumption of contaminatedfish and/orfish products (Ibrahim *et al.,* 2014). Bacteria associated with fish are broadly classified as non-pathogenic bacteria (e.g. *Clostridium botulinum, Salmonella* spp, *Listeria monocytogenes, Staphylococcus aureus, Shigella* spp. and *Escherichia coli (*Vasemägi *et al.,* 2017; Fernandes *et al.,* 2018), and pathogenic bacteria (e.g.*Vibrio* spp., *Vibrio parahaemolyticus, Aeromonas, and Yersinia. (*Elhadi *et al., 2016).*

Escherichia coli (E. coli) belongs to the family *Enterobacteriaceae.* It is a rod-shaped, Gram-negative bacterium which is commonly found in the lower intestine of most of the warm-blooded organisms (Jang, 2017). It has been found to be important pathogenic bacteria associated with freshly harvested or processed fish and are of significant global-public health problem, and a leading cause of most bacterial food-borne illnesses (Ibrahim *et al.,* 2014; WHO, 2019) with different disease syndromes including septic shock, meningitis, or urinary tract infections, gastroenteritis, bacteraemia, and typhoid fever and in severe

cases, death in humans (Meiyarasi *et. al.*, 2017). Among the *E. coli* species, the enterohemorrhagic *E. coli* (EHEC) is classified as a pathogenic strain because it produces Shiga toxins (Stxs), responsible for causing hemorrhagic colitis (HC) and Hemolytic Uremic Syndrome (HUS) in immunocompetent humans. Human diseases have been associated with the O26:H11, O91:H21, O111:H8, O157:NM, and O157 (Melton-Celsa, 1996; Paton, 1997) serotypes. *Escherichia coli*O157 has been identified as the most important serotype frequently associated with human infections. According to the WHO, 2019, *E. coli 0157* has been indicated as a causalagent for an estimate of 3,600 human hospitalizations in the US.

Consumption of contaminated food and water are the most frequent route of transmission while spread can also occur directly from person to person and from animal to person (Alam *et al.,* 2004). Recently, airborne transmission has been found to be a new route of transmission through contaminated buildings that contains animal exhibit (Varma *et al.,* 2003). The occurrence of this pathogen in fish is commonly related to its breeding, industrialization of environment andunhygienic practices(Fernandes *et al*., 2018). Fish contamination occurs through indiscriminate deposition of excreta and other wastes and unhygienic handling by human healthy carriers (Adedeji *et al.,* 2011and Fuhrimann *et al.,* 2015). Several studies have described the epidemiology and the prevalence of *E. coli* in fishranging between 4-90% (Adedeji *et al.*, 2011; Rafael *et al.,* 2014; Tiamiyu *et al.,* 2015 and Kikomeko *et al.,* 2016). These therefore, suggestthat microbiological standards for fish processing and marketing be established to ensureabsence of *E. coli* in foods in compliance with World Health Organization (WHO).

Antibiotic resistance and Multi Drug Resistance (MDR)global menace have continued to be on the rise with developing countries being worst affected; since most of these countries exhibit practices and attitudes that promote the development and spread of resistant microorganisms. This can be partly associated to the antibiotic prescriptions practices in many hospitals without confirmatory appraisal of the evidence of infections as well as identification of the causal agents (Prescott *et al.,* 1999). Furthermore, unaffordable laboratory tests and consultation fees, also have culminated in the present day practices of administration/prescription of broad spectrum antibiotics. This however results often to exposure to more bacteria, with resultant risks of dangerous side effects, super infections and the development of drug resistant mutants. Antibiotic therapy scheme for most *E. coli* infections includes third generation cephalosporin antibiotics, quinolones and macrolides. However, resistance and MDR to these drugs have been on the increase (Tiamiyu *et al.,* 2015 and Kikomeko *et al.,* 2016). Antimicrobial resistance (AMR) and the emergence of multiple drug resistant to *E. coli* (MDR) has become global public health concernrepresenting an increase in the severity of foodborne disease, leading to increased morbidity and/or mortalities of humans (Rafael *et al.,* 2014; Kikomeko *et al.,* 2016). Understanding the main risk factors

and how to reduce them is therefore essential for developing best management practice to safeguard public health. Therefore, this study was designed with a view to assess the presence of *E. coli* O157 in O. *niloticus* collected from different locations inIbadan, Nigeria.

II. MATERIALS AND METHODS

Study Area

Ibadan, Oyo state, Nigeria the largest city in West Africa, and the second largest in Africa, has land size of about 240km^2, situated at an average height of 200m above sea level, surrounded by secondary rainforest and savanna, and drained by three major river basins (Ogunpa, Ona and Ogbere). It is located on geographic grid reference longitude 3° 5E, latitude 7° 20N (Filani, 1994). It is an important region where intensive aquaculture and fishery activities form a central pivot.

Sampling Method and Sample Collection

Egbeda (A), Ibadan North West (B), Ido (C) and Ibadan North East (D) Local Government Areas (LGAs) in Ibadan were purposively selected based on the availability of *Oreochromis niloticus*. A total of fifty-two (52) apparently healthy (average weight 120.00±0.00gm) live tilapia comprising 16 each from the feral (A and B) and 10 each from cultured farms (C and D) were collected between July and August, 2019. Sampled tilapia were transported to the Food and Milk Hygiene Laboratory of the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan for bacteriological analysis. On arrival at the laboratory, fish were stored briefly and were properly identified (Chapman, 1992, FAO, 2016) and organs of interest were aseptically harvested using scalpel. 1gm of samples of gills, intestine and skin were collected and aseptically macerated with peptone water. A total of 156 samples consisting 52 of gill, intestine and skin samples were collected from the 52 *Oreochromis niloticus* for bacteriological analysis. Microbiological Standards and Guidelines by *European Union Reference Laboratory (EU, 2017) for isolation and characterization of E. coli O157* were used during the research work.The study was approved by the Animal Care and Use Research Ethics (ACUREC), University of Ibadan, Nigeria with approval number: UI-ACUREC/19/0079.

Isolation and Identification of Escherichia coli 0157

Isolation of*Escherichia coli 0157*was carried out according to the European Union Reference Laboratory (EU, 2017) for isolation and characterization of *E. coli O157*. Non-selective pre-enrichment was performed by aseptically harvesting 1gm of tissue sample, and then homogenised in 9 mls buffered peptone water (LabM®, UK) in a test-tube to give a dilution of 1:10. Test-tubes were corked properly, labeled and incubated overnight at 37°C. A 10 µl wire loop was used to pick a loop-full volume from the pre-enrichment broth and plated MacConkey Agar (LabM, UK) for Selective agar plating, and incubated at 37°C overnight. Colonies which

showedpinkish morphology suspected to be *E. coli* were sub-cultivated unto Sorbitol MacConkey Agar-(SMAC) (Oxoid^R) and incubated overnight at 37°C. The colonies appearing colourless on SMAC were selected as *E. coli O157,* and was followed by storage of pure cultures onto nutrient agar slants, incubated at 37°c overnight and stored in the fridge at 2^{0}C-8^{0}C

III. MORPHOLOGICAL AND BIOCHEMICAL TESTS

Morphological characteristics of isolates were performed through Gram staining. Biochemical characterization of isolates was performed by using Sugar fermentation tests, Catalase, Indole and Triple Sugar Iron agar(TSI) and then Incubated at 37°C overnight**.**

Antibiotic Susceptibility Test

The antimicrobial susceptibility test was carried out using the agar disk diffusion method as described by Bauer *et al*. (1966) and Clinical and Laboratory Standards Institute (CLSI) 2017 was used for the reading and interpretation of the zone of inhibition. Antibiotic sensitivity and resistance patterns of isolated *E. coli O157* were performed against 12 commonly used antibiotics belonging to different groups, using commercially available antibiotic discs (Biomark Lab®), containing antibiotics at different micrograms; Amikacin (AMK, 30μg), Cefotaxime (CTX, 30μg), Ceftazidime (CPZ, 30μg, Ceftriaxone (CTR, 30μg), Cefuroxime (CRX, 30μg), Chloramphenicol (CHL, 10μg), Ciprofloxacin (CIP, 5μg), Cotrimoxazole (COT, 25μg), Gentamicin (GEN, 10μg), Meropenem (MEM, 10μg), Tetracycline (TET, 10μg).

The inoculated plates containing the antibiotic discs were examined after 24hours of incubation at 37°C overnight. Diameters of the zones of inhibition were measured to the nearest millimetre, using ruler, which was held on the back of the inverted Petri plate. The Petri plate was held a few inches above a black, non-reflecting background and zones are measured in millimetre (mm) from the upper surface of the agar illuminated with reflected light, with the cover removed (EUCAST, 2015).

Data analysis

Data were analyzed using descriptive statistics and Chi-Square to test association between variables the prevalence among the groups.

IV. RESULTS

Identification of *Escherichia coli 0157*

From the one-hundred and fifty six (156) samples consisting 52 each of gill, intestine and skin samples collected from the 52 *Oreochromis niloticus*, *E. coli 0157* was isolated from a total of ninety-nine (99) samples. This represents an overall prevalence of 63.0%. The positive isolates comprised of thirty-three (33) gill, twenty (20) intestine and forty-six (46) skin samples representing 62.5%, 37.5% and 87.5%, respectively. The isolates based on location comprised 32(A), 32(B), 20(C), and 15(C). These assumedoverall location

prevalence were observed to be 65.4%, 65.4%, 66.7% and 50.0% for locations A, B, C and D, respectively.

Based on the locations, prevalence; 50.0%, 50.0% and 100.0% were observed for the gills, intestines and skin, respectively from the Location A(32: Gills -8; intestines-8 and skin-16); 100.0%, 0.0% and 100.0% were observed for the gills, intestines and skin, respectively from the Location B(32: gills -16; intestines-0 and skin-16); 50.0%, 50.0% and 100.0% were observed for the Gills, intestines and skin, respectively from the Location C(20: gills -5;intestines-5 and skin-10); and 50.0%, 50.0% and 50.0% were observed for the Gills, intestines and Skin, respectively from the Location D(15: gills -5;intestines-5 and skin-5).

A significant difference (p<0.05)was observed between the isolates obtained from the three (3) organs while. Isolates obtained from the four (4) locations showed no significant difference (p>0.05).
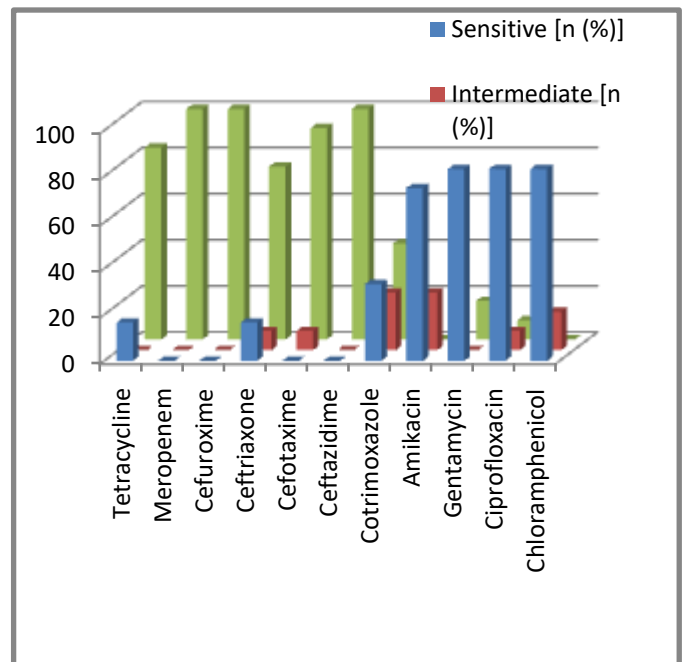

Fig 1: Histogram showing the resistance pattern of isolated *E. coli 0157* to commonly used antibiotics

Table 1: Multi-Drug Resistance Pattern for *Escherichia coli 0157*

Antibiotics	Number of Resistant Isolates (%)
CRX- CFZ-MEM	100.0
CRX-CTR-CTX-CFZ-MEM	83.3
CRX-CTR-CTX-CFZ-MEM-TET	66.7
CRX-CTR-CTX-CFZ-MEM-TET-COT	58.3
CRX-CTR-CTX-CFZ-MEM-TET-COT-GEN	8.3

Abbreviations: Amikacin (AMK, 30μg), Cefotaxime (CTX, 30μg), Ceftazidime (CPZ, 30μg, Ceftriaxone (CTR, 30μg), Cefuroxime (CRX, 30μg), Chloramphenicol (CHL, 10μg),

Ciprofloxacin (CIP, 5μg), Cotrimoxazole (COT, 25μg), Gentamicin (GEN, 10μg), Meropenem (MEM, 10μg), Tetracyclin (TET, 10μg).

Antibiotics belonging to different families were tested with each of the isolates. The different diameters obtained for each isolates were compared with the CLSI and EUCAST standards to determine whether they are resistant, intermediate or sensitive to each of the antibiotics.*Escherichia coli 0157* isolates tested showed highest resistance *(100%) to ceftazidime (CPZ), cefuroxime (CRX) and meropenem (mem)* while highest susceptibility *(83.3%)*was observed for*ciprofloxacin (CIP), chloramphenicol (CHL) and gentamycin (GEN)*as shown in Fig 1. Different levels of Multi-Drug Resistance (MDR) patterns were observed with 100% to the combination of CRX, CPZ, and MEM and least MDR of 8.3% to CRX-CTR-CTX-CFZ-MEM-TET-COT-GEN(Table 1).

V. DISCUSSION

Observed prevalence of *Escherichia coli 0157*in this study could be due to environmental factors and human activities such as defecation and refuse deposition in water bodies that play roles in contamination of water bodies which were observed at sampling locations. These observations are similar to those of Adedeji *et al.,* 2011; further affirming tilapia as a possible source of *E. coli*O157 to consumers and the consequential possible foodborne illness to consumers. *Escherichia coli 0157*isolated from *O. niloticus* (tilapia) in this study was observed to be 63.0% by conventional bacteriological. This report is similar to reports of Ibemenuga*et al.,* 2014 and Danba*et al.,* 2014 who both reported prevalence of 57.6% and 54.7%, respectively. However, the prevalence in this study is higher than the reported prevalence by Adebayo-Tayo*et al.,* 2012 (20.0%); and*Tiamiyu*et al.,* 2011 (24.0%). Pathogenic *E. coli*can cause foodborne disease, ranging from mild enteritis to serious illness and death. *E. coli* causes diarrhea, urinary tract and kidney infections and peritonitis septiceamia (Meiyarasi *et. al.*, 2017). Where animal manure is used as pond fertilizers, there is a risk that pathogenic strains of *E. coli* may be present in pond water leading to the occurrence of waterborne human infection caused by *E. coli* 0157; hence a potential risk to public health where bovine manure is used as pond fertilizer (WHO, 1997; Rafael *et al.,* 2014; Tiamiyu*et al.,* 2015 and Kikomeko *et al.,* 2016).The isolation of *E. coli* 0157 from fish samples in this study is worrisome because of their potential in causing ill-health in human upon consumption. These pathogens might have been introduced into the production systems through indiscriminate deposition of excreta and other wastes as well as through unhygienic handling by human healthy carriers as affirmed by Tiamiyu*et al.,* 2011 and Fuhrimann*et al.,* 2015.Fish as an important source of animal protein for human diet (Ayanda *et al.,*2018) are susceptible to variety of bacteria through human activities, unhygienic practices, industrialization etc (Adedeji *et al*., 2012), as well as subsequent contamination of fish and fish products during storage and improper handling or cooking, thereby portending public health hazards to humans(Seel*et al.,* 2016)

Resistance of bacteria to antimicrobial agents and the MDR are a reflection of misuse or abuse of these antibiotics in the sampled fish, as well as indiscriminate use and disposal of antibiotics in the environment, posing health hazards to humans (Okpo*et al.,* 2016). A 100% resistance by *E. coli* O157 isolated from *O. niloticus* (tilapia) in this study was observed for Ceftazidime, Meropenem and Cefuroxime and susceptibility to Ciprofloxacin, Chloramphenicol and Gentamicin were observed in this study. The observed sensitivity of *E. coli* O157 to Gentamicin and resistance to Cefuroxime, Cotrimoxazole and Tetracycline are similar to reports of Adedeji *et al.,* 2011; Tiamiyu*et al.,* 2015 and Kikomeko *et al.,* 2016 who all reported E. coli O157 to be sensitive to Gentamicin and resistant to Cefuroxime, Cotrimoxazole and Tetracycline. However, this report contrasts the report of the resistance of *E. coli* to Ciprofloxacin and Chloramphenicol by Adedeji *et al.,* 2011. The resistance and susceptibility profile observed from this study are also partly similar to the findings of Adedeji *et al.*, 2012; Rafael *et al.,* 2014; Musefiu and Olasunkanmi, 2015 and Kikomeko *et al.,* 2016.The Antibiotic sensitivity tests conducted on the isolated *E. coli* obtained from *O. niloticus*in this study revealed multiple drug resistance of *E. coli* to 3-8 antibiotics especially to those commonly used in fish farms such as cotrimoxazole and tetracycline. These observations are similar to reports of Rafael *et al.,* 2014 and Tiamiyu*et al.,* 2015.The observed MDR in this study evidences this pathogen as multi resistant bacterium in nature, representing a potential threat to human and public health. MDR is a global public health problem associated with outbreak of major global epidemics (Crump *et al.,* 2015). This report therefore confirms *E. coli* resistance to antibiotics evidencing transfer risks of resistant bacteria and genes to human through consumption of aquaculture products (Kikomeko*et al.,* 2016). Coincidentally, the resisted antibiotics are found to be those categories and types commonly used for treatment of various ailments in humans. This rather unfortunate incidence therefore supports the theories of antibiotics' introduction into the aquatic ecosystems from humans as described by Rafael *et al.,* 2014 and further reinforced by the reports of Owino, 2014 and Fuhrimann*et al.,* 2015. The observed antibiotic resistance and MDR in this study therefore support hypothesis about the rapidly increasing menaces of AMR and MDR globally and a global public health problem associated with outbreak of major global epidemics (Levy *et al.,* 2001 and Crump *et al.,* 2015) and resultant drug side effects, super infections and development of drug resistant genes and mutants genes (Kikomeko*et al.,* 2016).

VI. CONCLUSION

1. This study revealed thattilapia obtained from Ibadan are contaminated with *Escherichia coli 0157 which is a* pathogen of public health importance, hence, could be transmitted to humans upon consumption.

2. This study also elucidated the multi-drug resistant characteristics of *E. coli0157* isolates fromtilapia obtained from Ibadan; this could lead to transfer of resistant bacteria and genes to human from handling and/or consumption of contaminated fish and from the environment.

VII. RECOMMENDATIONS

- This study confirmed the occurrence of *E. coli* in the study areas and since thispathogen is of public health importance, awareness should be created among fish farmers and stakeholders on possible health hazards accompanying handling and/or consumption of fresh and undercooked fish.

- A comprehensive epidemiological study of *E. coli* in Nigeria is highly recommended, and systems for monitoring contamination levels of other zoonotic pathogens should be instituted into the aquatic value chain.

- Further researches and studies of aquatic animal health and zoonosis should be adapted and employed as part of the tools for controlling diseases of public health importance.

- Regulatory policies and standard operating practices for hygienic practices in the fishing and aquatic environments, controlled human activities, fishing procedures and handling of fish should be instituted and be followed by fishers and farmers to avoid disease outbreaks.

CONFLICTS OF INTEREST

Authors have no conflict of interest.

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