Incidence and Antibiotics Profile of Isolated Escherichia Coli 0157 from Meat and Fish in Kwali Area Council Area of F.C.T.

Alu A. J.[1], Omeiza G. K.[2], Ameh J. A.[3]

1. **Faculty of Agriculture, University of Abuja.**
2. **Veterinary Public Health, University of Abuja.**
3. **Veterinary Microbiology, Veterinary Teaching Hospital, University of Abuja.**

ABSTRACT

The emergence of O157 strains of *Escherichia coli* poses serious threat to public health with regards to their devastating and a zoonotic importance. *Escherichia coli* 0157 is a highly virulent Enterohemorrhagic *Escherichia Coli* (EHEC), an acronym used to denote a sub-set of of Shiga toxin (Stx)-producing *E. coli* (STEC), which is also known as verotoxin-producing *E. coli* (VTEC), that causes severe diseases in humans including hemorrhagic colitis, bloody diarrhea and the hemolytic uremic syndrome (HUS). The present investigation was carried out to study the incidence and antibiotic profile of *E. coli* O157 strains isolated from raw meat, frozen fish, and cat fish samples in Kwali Area Council of F.C.T. A total of Ninety-six meat and fish samples were collected. Samples were stomached then isolation and identification of *E. coli* O157 was carried out using selective enrichment, plating on selective and specific medium (SMAC) and Latex agglutination test kit was used to confirm *E. coli* O157 isolates. Eighteen out of Ninety-six meat and fish samples were positive for sorbitol negative strains of *E. coli* (8.94%). The strains reactive to the agglutination test were all non-sorbitol fermenters. The overall Prevalence of *E. coli* O157 strains in the cow meat and intestine sampled was 10.94%, in Shawa fish skins (8.34%), Mackerel Titus fish intestine and gills (12.26%), and the prevalence of *E. coli* O157 in Cat fish intestine and skin was higher than the other samples, which was positively associated ($p < 0.05$) with presence in the cow samples (18.30%). The *Escherichia coli* O157 strains from meat were only susceptible to Ampicillin (83%), and were 83% resistance to Amoxicillin, Sulphamethaxazole/Trimethoprim, Nitrofurantoin and 66.7% resistance to Cefoxilin, Tetracycline, Gentamycin. The fish isolates shows Erythromycin, Ampicillin, Nitrofurantoin (100%) resistance, Sulphamethaxazole/Trimethoprim 75% resistance, Amoxicillin, Tetracycline and Streptomycin showing 58% resistance, while Amikacin (58%), Ciprofloxacin, and Gentamycin (50%) susceptible. The antibiotic resistant *E. coli* 0157 found in this study are of public health significant.

1.0 INTRODUCTION

E. coli a Gram negative rod, a facultative anaerobe found in the GI tract of mammals, belongs to the family Enterobacteriaceae (Marin *et al.* 2009). Most *E. coli* are harmless and actually are important members of a healthy human intestinal microbial community. However, *E. coli* is a very diverse group of bacteria consisting of many ecotypes (the harmless variety) and pathotypes. The pathogenic strains of *E. coli* can cause diarrheal diseases, urinary tract infections, and even meningitis which are transmitted via contaminated food or water, or through contact with infected animals or people. *Escherichia coli* 0157 is a highly virulent Enterohemorrhagic *Escherichia Coli* (EHEC), an acronym used to denote a sub-set of Shiga toxin (Stx)-producing *E. coli* (STEC), which is also known as verotoxin-producing *E. coli* (VTEC), that causes severe diseases in humans. *E. coli* 0157 was first reported by the Centers for Disease Control in 1982 (CDC, 1982)

being isolated from hamburger and cattle which are considered to be the primary reservoir for EHEC 0157, (Hancock, *et al.* 1994). Animal production, in the developing countries has faced challenges from infections due to different types of microorganisms. In many attempts to curb the menace of loss in production due to diminished crop yield, applications of antimicrobials have gained increased attention. Such practices are not without consequence which may include hypersensitivity reaction and development of resistant strains of microbes that can be shared between man and animals. There are identified principles of mechanisms underlying the development of resistant strains of microorganisms, (Ratnam *et al.*, 1988), (al-Saigh *et al.*, 2004). Meat and Fish are the most popular and nutritious components of human diet and are also important sources of food-borne pathogens (Abdullahi *et al*., 2006). In terms of meat and fish consumption, beef and its offal, Cat fish, Markerel (Titus) and Herrings (Shawa) iced fishes are the most commonly consumed in urban areas of Nigeria (Obayelu *et al*., 2009). Fish and meat has traditionally been part of the human diet in many countries (Huss, 1993) and they are important sources of nutrients, especially of high digestible proteins (Faber, *et al.*, 2010). Fish constitute the cheapest source of animal protein in Africa (Clucas and Ward, 1996). Meat or fish contaminated with microorganism such as *E.coli* 0157 becomes unwholesome and can lead to serious food-borne disease outbreak. The quality of fish or meat is strongly determined by the level of its bacterial microbiota (Gram, 1992), the use of *E. coli* as a sanitary indicator for fish and meat has been first reported in the 1930s (Griffiths and Fuller, 1936) and had been widely applied as a microbiological quality parameter, especially where fecal contamination is concerned (Baer, *et al.* 1976, Silva and Hofer, 1993).

MATERIALS AND METHODS

The equipment and materials used for this study include:

A. Materials/Equipment

- Incubator (BIO-RAD Laboratories, USA)
- Electronic Heating block (WEALTEC Corp)
- Refrigerator (African Advice.Com)
- Autoclave (Fedegari Autoclavi SpA, Italy)
- Weighing Balance (Scale Tech. South Africa)
- Hot-air Oven (Sree Valsa Engineering Company, India)
- Centrifuge (Beijing Era Beili Centrifuge Co; Ltd, BEILI)
- Pipette Adjustable (Qindao Hiprove Medical Technologies Co; Ltd. China)
- Microscope (HOVER LABS^{TM} India)

B. Media and Supplements

- Luria-Bertani Broth (Inqaba Biotec, South Africa)
- Nutrient Agar (Merck KGaA, Darmstadt, Germany)
- Sorbitol MacConkey Agar & Cefixime-Tellurite (Oxoid, CM813, Oxoid SR 172)
- MethylRed-VogesProskauer,TSB,SCA&PeptoneWater(Ipswich,UnitedKingdom)

C. Reagents

- Ethanol (Flukai Sigma-Alduch Chemie Gmbti Buscits Switzerland)
- Kovac's Reagent, Potassium iodide, Iodine crystal, Crystal violet and Safranin (Himedia Laboratories Pvt. Ltd, India)
- Methyl Red, Potassium Hydroxide (KOH) (BDH laboratory England)

D. Glasswares

- Petri dishes, Bijou bottles, Test tubes, Conical flask, Measuring cylinder & Beakers

F. Test Kit

- *E. coli*0157 Latex Testkit & Antibiotics Susceptibility Discs (Oxiod Ltd, Wade Road Basingstoke Hants, UK)

METHODS (Study Area, Sampling, Laboratory Procedure)

The study was carried out in Kwali Area Council, Abuja, F.C.T. Simple random sampling technique was employed in selecting the markets in the Area Council. The total of ninety-six (96) meat and fish samples were collected for this study.

Meat and Fish samples were transported to the laboratory and processed for analysis within 2 to 6 hours of collection. The media were prepared based on the manufacturer's instructions. Isolation, Identification and Characterization of *E. coli* 0157 was done by inoculating 1ml prepared sample broth culture onto specific and selective media (MacConkey, Eosin Methylene Blue Agar and TSMAC), and incubated for 18 – 24 hours at 35 – 37ºC. Each colonial morphology and reactions on agar media, like colony size, consistency, shape and pigmentation, lactose and sorbitol fermentation, were noted while the colonies were gram stained, and their motility tested. Biochemical tests were performed on presumptive *E. coli* colonies and other isolates using standard techniques (Cheesbrough, 2006). All non-sorbitol-fermenting *E.coli* colonies were confirmed by testing for agglutination with O157 latex reagents to determine if the isolates belonged to the O157 serogroup (Nataro and Kaper, 1998).

Antibacterial susceptibility testing

The antimicrobial susceptibility was performed following the standard agar disk diffusion method according to CLSI (2008), using 12 commercially available antimicrobial discs. Each isolated bacterial colony from pure fresh culture was transferred into a test tube of 5 ml Tryptone Soy Broth and incubated at 37°C for 6 hrs. Mueller-Hinton agar plates were prepared according to the manufacturer's guidelines. A sterile cotton swab was immersed into the suspension and then swabbed in three directions uniformly on the surface of Mueller-Hinton agar plates. Antibiotic discs were placed and pressed on the inoculated plates using sterile forceps and incubated at 37°C for 24 hrs. The diameter zone of inhibition formed around each disk was measured using a black surface, reflected light and transparent ruler by laying it over the plates. The results were classified as sensitive, intermediate, and resistant according to the standardized table supplied by the manufacturer (CLSI 2008).

RESULTS AND DISCUSSION

All the Ninety-six, 96 (100%) meat and fish sampled, comprising of 128 Meat and 170 Fish sampled, were contaminated; 84 (87.5%) had bacteria colonies that could be counted ≤ 300 while 12 (12.5%) of the samples collected produced colonies that were too numerous to count (TNTC) >300. The reason could be due to the fact that meat and fish serves as suitable medium for bacteria growth/activities, possible sources of contaminations could be during processing and handling of the products. Water can also be a major source of meat contamination, especially in cases where the meat is washed with river water, and catfish harvested from river could as well be contaminated with fecal matter, Chen *et. al*, (1985). The result shows that meat had higher microbial load (6.76E+14cfu) than fish (4.32E+14cfu). The relationship was however not statistically significant (t=0.143, p=0.887). The total coliform count from the study indicates the highest coliform count in fish to be $1.30x10^{15}$ and in meat to be $4.50x10^{15}$. The Meat were highly contaminated by coliform bacteria with the overall mean Coliform Counts of $2.49x10^{14}$ cfu/ml while the Fish yielded overall mean Coliform Counts of $9.77x10^{13}$ cfu/ml. From the total of 34 Meat samples examined, 20 (58.8%) were positive for *E. coli*, while out of 62 Fish samples examined, 22

(35.4%) were positive for *E. coli*. Totalling 42 (43.8%) meat and fish samples positive for *E. coli*. The variation between meat and fish was statically significant (P>0.05). Out of the *E.coli* positives, 18 isolates (42.9%) were confirmed serologically with the oxiod agglutination test kit to be positive for *E. coli 0157*, this is made up of 6 meat and 12 fish. In contrast, the prevalence of *E. coli* O157 in beef in this study was higher than the 8.8% prevalence reported by Abong (2008) in South Africa and Hajian *et al.* (2011) in Iran; and lower than 53% prevalence reported by Dahiru *et al.* (2008), in fresh beef meat in Nigeria.

The antibiotic profile of *E. coli* 0157 isolates in this study shows resistance to a wide range of antibiotics and susceptible to certain antibiotics. The results indicates that; Erythromycin, Amoxicillin, Sulphamethaxazole/Trimethoprim, Nitrofurantoin were 83% and Cefoxilin, Tetracycline, Gentamycin were 66.7% resistance and Ciprofloxacin, Amikacin, chloramphenicol are showing 50% intermediate resistance while only Ampicillin showing 83% sensitive in meat isolates. Also in the fish isolates, Erythromycin, Ampicillin, Nitrofurantoin are showing 100% resistance, Sulphamethaxazole/Trimethoprim 75% resistance, Amoxicillin, Tetracycline and Streptomycin showing 58% resistance, Chloramphenicol showing 41% intermediate resistance while Amikacin is showing 58% sensitive, Ciprofloxacin, and Gentamycin showing 50% sensitive. The results agree with similar work done by Momtaz *et. al*, (2012), who observed that nine strains (15.78%) of *E. coli* has been found to be resistant to a single antimicrobial agent (Tetracycline) and 11 strains (19.29%) resistance to two antimicrobial agents (Sulfonamides and Erythromycin) and 64.91% being multi-resistant. However, contrary to the study by Sami *et. al*, (2014), who identified that out of 45 *E. coli* isolates, 11.1% are *E. coli* O157 and all are sensitive to commercial antibiotics.

CONCLUSION

From this study, the data obtained showed that culturable *Escherichia coli O157* were isolated from meat and fish obtained from Kwali markets in Abuja, F.C.T. The isolation of *E. coli* O157 pathogen in Kwali, urban area is an indication that contaminated meat and fish can be sold to buyers and the consumers of meat and fish from the markets, which agrees with a similar study by Nkaga and Uriah, (1981). This also agrees with various studies that identified cattle to be a major reservoir of *E. coli* O157:H7 (Hancock et al., 1994). Human diseases caused by *E. coli* O157 can be linked directly or indirectly to cattle (Dany et al., 2007). These shows further that meat and fish hygiene handling practices remain a great challenge.

The antibiotic multi-resistance in this work, indicates the misuse or overuse of antimicrobial agents in different fields, such as human, agriculture and veterinary medicine as prophylactic or growth promoting agents in the food animal, which has created enormous pressure of antimicrobial resistance among bacterial pathogen and endogenous micro flora as observed by WHO (1997), this has a direct clinical implication which involved the development and transfer of resistant gene by these bacteria, thereby making it difficult to effectively control bacterial infections (Karlowsky *et al*., 2003). The occurrence of *E. coli* O157 in raw meat and fish, and the existence of antimicrobial resistant isolates highlight the potential threat to public health. Hence implementations of *E. coli* O157 prevention and control strategies from sources, handling and retailing of meat and fish to consumption of same are crucial.

REFERENCE

Abdullahi, I. O., Umoh, V.J., Ameh, J. B. and Galadima, M. (2006). Some hazards associated with the production of a popular roasted meat ('tsire') Zaria, Nigeria, Food Control, 17: 348-352.

Abong B. O., (2008). Prevalence and potential link between E. coli O157:H7 isolated from drinking water, meat and vegetables and stools of diarrhoeic confirmed and non-confirmed HIV/AIDS patients in the Amathole District - South Africa. J Appl Microbiol. 2008 Aug;105(2):424-31. doi: 10.1111/j.1365-2672.2008.03756.x. Epub 2008 Feb 19

Al-Saigh H. Zweifel C. Blanco J. Blanco JE. Blanco M. Usera MA. Stephan R. Fecal shedding of Escherichia coli O157, Salmonella, and Campylobacter in Swiss cattle at slaughter. J Food Prot. 2004;67:679–684.

Baer, E.F., Duran, A.P., Leininger, H.V., Read Jr., R.B., Schwab, A.H. and Swartzentruber, A. (1976) Microbiolo- gical quality of frozen breaded fish and shellfish products. Applied and Environmental Microbiology, 31, 337-341.

Dany J., Bhat M, Eckmann K., (2007). Outbreak of Escherichia coli O157:H7 associated with raw milk consumption in the Pacific Northwest. Foodborne Pathog Dis. 2008 Jun;5(3):321-8. doi: 10.1089/fpd.2007.0072.

Centers for Disease Control (CDC). Isolation of E. coli O157:H7 from sporadic cases of hemorrhagic colitis - United States. MMWR Morb Mortal Wkly Rep. 1982 Nov 5;31(43):580, 585. PubMed PMID: 6817062.

Chapman, P.A., Siddons, C.A., Cerdan Malo, A.T. and Harkin, M.A. (1997). A 1-year study of Escherichia coli O157 in cattle, sheep, pigs and poultry. Epidemiology and Infection119, 245–250.

CLSI (2008), Performance Standards for Antimicrobial Susceptibility Testing; Eighteenth Informational Supplement. Wayne, Pennsylvania, USA: CLSI; 2008

Clucas I. J, Ward A. R., (1996). Post Harvest Fisheries Development. A Guide to Handling, Preservation, Processing and Qauility. Chatham Maritime, Kent ME4TB, United Kingdom. Pp. 665.

Cheesbrough, M., 2006. District Laboratory Practice in Tropical Countries. 2nd Edn., Cambridge University Press, Cambridge, UK., ISBN-13: 9781139449298. Chen, H. and D.G. Hoover, 2003. Bacteriocins and their food applications. Comprehen. Rev. Food Sci. Food Saf., 2: 82-100.

Chen J., Hsing Chen, Journal of Food and Drug Analysis, 1985, 13 (3). pp 56-78.

Dahiru, M., Uraih, N., Enabulele, S.A and Shamsudeen, U., (2008). The prevalence of e. coli 0157;H7 in fresh and roasted beef in Kano city, Nigeria. Bayero Journal of Pure and Applied Sciences, 1(1):39 – 42.

Faber, T.A., Hernot, D.C., Parsons, C.M., Swanson, K.S., Smiley, S., Bechtel, P.J. and Fahey Jr., G.C. (2010) Pro-tein digestibility evaluations of meat and fish substrates using laboratory, avian, and ileal cannulated dog assays. Journal of Animal Science, 88, 1421-1432. doi:10.2527/jas.2009-2140

Gram, L. (1992) Evaluation of the bacteriological quality of seafood. International Journal of Food Microbiology, 16, 25-39. doi:10.1016/0168-1605(92)90123-K

Griffiths, F.P. and Fuller J.E. (1936) Detection and sig-nificance of Escherichia coli in commercial fish and fil-lets. American Journal of Public Health and the Nations Health, 26, 259-264. doi:10.2105/AJPH.26.3.259

Hajian S, Rahimi E, Mommtaz H: *A 3-year study of Escherichia coli O157:H7 in cattle, camel, sheep, goat, chicken and beef minced meat in Proceedings of the International Conference on Food Engineering and Biotechnology (IPCBEE '11)*. IACSIT Press, Singapoore; 2011.

Hancock, D. D, Besser, T. E, Kinsel, M. L, Tarr, P. I, Rice, D. H, Paros, M. G., (1994). The prevalence of Escherichia coli O157.H7 in dairy and beef cattle in Washington State. Epidemiology Infectious Disease, 1994 Oct;113(2):199-207. PubMed PMID: 7925659.

Huss, H.H. (1993) Assurance of seafood quality. FAO Fisheries Technical Paper No. 334, FAO, Rome, 169 p.

Karlowsky J. A., Thornsberry C., Jones M. E., Sahm D. F. 2003. Susceptibility of antimicrobial-resistant urinary Escherichia coli isolates to fluoroquinolones and nitrofurantoin. Clin. Infect. Dis. 36:183–187

Marín, A.; Casas-Valdez, M.; Carrillo, S.; Hernandez, H.; Monroy, A.; Sangines, L.; Perez-Gil, F., 2009. The marine algae *Sargassum spp.* (Sargassaceae) as feed for sheep in tropical and subtropical regions. Rev. Biol. Trop., 57 (4): 1271-1281

Momtaz H, Rahimi E, Moshkelani S Veterinarni Medicina, 2012, 57(4), pp 193–197.

Nataro, J.P. and Kaper, J.B. (1998) Diarrheagenic Esche- richia coli. Clinical Microbiology Reviews, 11, 142-201.

Nkanga, E.J., Uriah, N. (1981). Prevalence of Staphylococcus aureus in meat samples from traditional markets in Benin City, Nigeria and possible control by use of condiments. Journal of Food Protection.44:4-8.

Obayelu, A.E., Okoruwa, V.O. and Oni, O.A. (2009). Analysis of rural and urban households' food consumption differential in the North-Central, Nigeria. Journal of Development and Agricultural Economics 1(2): 18-26.

Ratnam S. March SB. Ahmed R. Bezanson GS. Kasatiya S. Characterization of Escherichia coli serotype O157:H7. J Clin Microbiol. 1988;26:2006–2012.

Sami A. Abdaslam, Mohamed A Hassan, Hazem H, Kaheel, Talib M, Abobaker, Tassneem H, Alnourain Hamid A, Hamdan S, Gokul Shanka, John J Thambirajah, Curr Res in Microbio and Biotech, 2014, 2(3), pp 391-397.

Silva, A.A.L. and Hofer, E. (1993) Resistance to antibio- tics and heavy metals in Escherichia coli from marine fish. Environmental Toxicology and Water Quality,8,1-11. doi:10.1002/.2530080102

World Health Organization (WHO) (1997). Food safety: outbreak of Escherichia coli 157 infection. Weekly Epidemiologic Record. 3.