NIGERIAN VETERINARY JOURNAL

ISSN 0331-3026

Nig. Vet. J., March 2019 Vol 40 (1): 86 - 94.

https://dx.doi.org/10.4314/nvj.v40i1.8 **ORIGINAL ARTICLE**

Isolation of *Escherichia coli* O157:H7 in Poultry by Culture, Serology and Polymerase Chain Reaction Technique in Jos, North Central Nigeria

Buharshak, O.H*., Egah, D.Z, Onyedibe, K.

Department of Medical Microbiology, Faculty of Medical Sciences, University of Jos.
*Corresponding author: Email: otriinshak@gmail.com, Tel No: +234 8033214005

SUMMARY

Escherichia coli O157:H7 is an important human pathogen capable of causing food borne infections with severe consequences of haemorrhagic colitis (HC), haemolytic uraemic syndrome (HUS) and haemolytic thrombocytopaenic purpura. Information about isolation and characterisation of the organism in poultry in North Central Nigeria is lacking. An isolation and characterisation of E. coli O157:H7 was carried out in four hundred and thirty- three (433) samples (167 fresh faeces, 217 cloacal and 49 laryngeal swabs) from intensively reared chicken from 20 farms in Jos, Central Nigeria. Microbiological culture, serology and polymerase chain reaction (PCR) methods were used. Out of the 433 samples, 26 (6%) of the isolates were phenotypically identified as E. coli O157:H7 on cefixime-tellurite Sorbitol MacConkey (CT-SMAC) agar. None of the isolates was positive by serology using Wellcolex* Escherichia coli O157:H7 kit R30959601 (Remel Europe Ltd, Dartford Kent UK) and for rfbEO157 and fliCh7; the defining genes for E. coli O157:H7 serotype on further molecular analysis. Of the 26 isolates, 5 (19.2%) were from fresh faeces while 19 (73.1%) and 2 (7.7%) were from cloacal and laryngeal swabs respectively. Although E. coli O157:H7 was phenotypically found, we could not ascertain if intensively reared chicken in Jos habour E. coli O157:H7 based on the absence of serotype specific genes rfbEO157 and fliCh7 by PCR analysis.

Key words: Escherichia coli O157:H7, poultry, polymerase chain reaction (PCR), cefixime-tellurite Sorbitol MacConkey (CT-SMAC) agar.

INTRODUCTION

Escherichia coli O157:H7; a member of the verocytotoxin-producing *E. coli* family has been identified as a food borne pathogen capable of causing serious morbidity and mortality (Wani *et al*., 2004). Severe diarrhea resulting from infection with *E. coli* O157:H7 may lead to life threatening complications including haemorrhagic colitis (HC), haemolytic uraemic syndrome (HUS) and haemolytic thrombocytopaenic purpura (Wang *et al*., 2014). Over 200 outbreaks of *E. coli* O157:H7 associated infections have been reported in 30 countries worldwide (Doyle *et al*., 2006; Mead and Griffin,

1998). Contaminated foods and water are major source of infection especially foods of bovine origin (Nataro and Kaper, 1998). Other vehicles of transmission include contaminated milk and milk products, unpasteurized juice, raw vegetables such as lettuce, raddish sprouts, alfalfa sprouts, spinach, cucumber, cabbage and bitter leaf (Michino *et al*., 1999; Reuben and Makut, 2014; Albarri *et al*., 2017).

Ruminants especially cattle have been established as major reservoir of *E. coli* O157:H7 (Witold and Carolyn, 2011). In addition, some earlier studies (Dipineto *et al*., 2006; Kalin *et al*., 2012) reported isolation of *E. coli* O157:H7 in living layer hens and post mortem samples of broiler liver and caecum and from contaminated poultry meat and faeces (Olatoye *et al*., 2012; Aibinu *et al*., 2007). Some of the isolates from the aforementioned studies possessed *hlyA* (enterohaemolysin) and *eaeA* (intimin) genes which are known virulence factors of human pathogenic strains; bringing to the fore public health concerns of cross transmission (Wang *et al*., 2002; Rwego *et al*., 2008). The increasing use of untreated organic poultry manure to fertilize fish ponds, vegetable and crop farms in the area of study coupled with the ability of *E. coli* O157:H7 to survive in low acidic pH and the requirement for low infective dose (Nataro and Kaper, 1998) to cause an infection warrants an investigation into the role of poultry in the epidemiology and human transmission of *E. coli* O157:H7. There are limited reports of isolation and characterization of *E. coli* O157:H7 in poultry especially in Nigeria. In addition, most poultry farms in the study area are located within or near residential quarters and in close proximity to rivulets which serve many purposes to the nearby dwellers including as drinking water or for cooking.

The aim of this study was to determine the presence of *E. coli* O157:H7 in intensively reared chickens in Jos, North Central Nigeria and to investigate the isolates for the presence of some *E. coli* O157:H7 virulence associated genes (*stx1*, *stx2*, *hlyA* and *eaeA*).

MATERIALS AND METHODS

Study Area: Jos is located in Plateau State, central Nigeria on an elevation of 1,217m (3,993 ft) above sea level between latitude 90 48' 00'N and 80 52'00'E. It has a land area of about 8,600km2 or 860,000 hectares and a population of about 900,000 people (based on 2006 national census). Its weather is suitable for poultry, livestock, crop and vegetable farming. Poultry farming is a mainstay of many families both as a hubby and on commercial basis located within and close to residential areas, rivulets and ponds.

Sample Collection: Four hundred and thirty-three (433) samples (167 or 38.6% of freshly voided faeces, 217 or 50.1% cloacal and 49 or 11.3% laryngeal swabs) from apparently healthy intensively reared chickens were collected from 20 farms by a random sampling method from the different geographical locations within Jos between May and October, 2015. Samples were preserved on ice at 4 0C in a cooler and transported to the laboratory within 1 to 2hrs.

Sample processing:

In the Laboratory, 25g of faecal sample was enriched in 225ml modified tryptone soy broth (mTSB) by supplementation with novobiocin 10mg/L (Oxoid Ltd, Hamshire, England). Each swab sample was also enriched in 9ml of mTSB. A positive control; E. coli O157:H7 (LMG 21756 or ATCC 700728) was reconstituted in nutrient broth (Oxoid CM3) as recommended by the manufacturer (BCCM). Samples were incubated aerobically for 18-24hrs at 37 0C.

A loopful of each enriched sample was streaked on Sorbitol MacConkey agar supplemented with Cefixime-Tellurite (2.5mg and 0.05mg/L) (Oxoid Ltd, Hamshirre, England) (CT-SMAC) and incubated aerobically for 18-24hrs at 37 0C. Non sorbitol fermenters which appeared slightly transparent, almost colourless with a weak brownish appearance were selected and plated on nutrient agar slant, incubated at 37 0C for 24hrs and stored at 4 0C for further tests (March and Ratman, 1986; Zadik et al., 1993). The isolates

were biochemically screened for indole production, hydrogen sulfide production in triple sugar iron (TSI) agar, citrate utilisation, urease production, acid production on inositol and acid and gas production from glucose and for motility on sulfide indole motility medium. Positive and negative controls were processed using same protocol.

Serology

All CT-SMAC negative isolates and non-sorbitol (NSF), non-lactose fermenting (NLF) isolates confirmed as E. coli biochemically were screened serologically using Wellcolex* Escherichia coli O157:H7 kit R30959601 (Remel Europe Ltd, Dartford Kent, UK). Wellcolex* E. coli O157:H7 kit is a rapid latex agglutination test for the presumptive identification of Escherichia coli O157:H7 isolates on laboratory media. The test contains two test reagents. The somatic (O157) antigen test reagent which consists of red latex particles coated with antibodies specific for E. coli O157 (O) antigen. When a drop of the reagent is mixed on a card with a suspension of E. coli O157 organisms, positive test is indicated by rapid agglutination of specific IgG and O157 lipopolysaccharide antigen. The H7 test reagent also consists of blue latex coated with antibodies specific for the flagellum antigen; (H7) antigen. The agglutination reaction was controlled by use of normal saline on a drop of emulsified fresh colony grown on nutrient agar (Oxoid). Positive and negative control kits are provided in the Wellcolex* Escherichia coli kit R30959601 (Remel Europe Ltd, Darford Kent, UK). The instruction of the manufacturers on choice of media and laboratory practice was followed strictly.

DNA Extraction

Three (3) colonies of the non-sorbitol fermenters phenotypically identified as E. coli O157:H7 were sub-cultured into mTSB and incubated for 24hrs at 37 0C. The positive control strain of E. coli O157:H7 was also processed in parallel at the same time. DNA was extracted from 3ml of broth using ZR Fungal/Bacterial DNA KitTM (Catalog No D6005) according to the manufacturer's instruction. The eluted DNA was stored at -80 0C until ready for PCR analysis. Genes, base sequences and predicted sizes of amplified products for the specific oligonucleotide primers used in this study are shown in Table I.

TABLE I: Genes, sequences and amplicon sizes of primers used in investigating E*scherichia. coli* O157:H7 in Jos, Nigeria

Genes	Sequence (5´ to 3´)	Size of PCR amplicon (bp)	Reference
16S rRNA	F- CCCCCTGGACGAAGACTGAC R- ACCGCTGGCAACAAAGGATA	401	Wang *et al*.,(2002)
*rfbE*O157	F- CTACAGGTGAAGGTGGAATGG R- ATTCCTCTCTTTCCTCTGCGG	327	Wang *et al*.,(2002)
*fliC*h7	F-TACCATCGCAAAAGCAACTCC R- GTCGGCAACGTTAGTGATACC	247	Wang *et al.*,(2002)

Multiplex Polymerase Chain Reaction (mPCR)

Primers (Table 1) used for this reaction were as designed by Wang *et al.*, (2002) and supplied by Inqaba SA. *E16S rRNA* was added as internal control. Positive control used for this reaction was also *E. coli* O157:H7 non toxigenic strain LMG 21756 while sterile nuclease free water (Biolabs SA) served as negative control.

Amplification of bacterial DNA was performed in GeneAmp® 9700 (Applied Biosystems) using 25µl volume of master mix containing 3µl of prepared sample supernatant; 2.5µl of 10X PCR buffer, 1.5µl Magnesium chloride, 0.125µl of each of the four deoxynucleotide triphosphates (dNTPs), 0.3µl *fliC*h7, 0.9µl *rfbEO157*, 0.2µl *E16SrRNA* and 0.25µl Taq polymerase at an initial denaturation temperature of 95 0C for 8mins. This was followed by 30 cycles of denaturation at 95 0C for 30sec; annealing at 58

0C for 30sec and extended at 72 0C for 30sec. Final extension was at 72 0C for 7min. The amplified products were visualized by electrophoresing 10µl of the amplicons in 1X agarose gel (1.5% 1X TBE Electrophoresis Buffer; Fermenters®) stained with 5µl of 10mg/ml ethidium bromide (Promega) at 130 volts for 45min (Bio-Rad); 50 lanes DNA molecular ladder (Fermenters®) was used as marker. The amplified DNA fragments were visualized through U.V. transilluminator (Sigma®) and results were documented using GelDoc™ XRT Image Lab™ software Documentation System (Bio-Rad).

STATISTICAL ANALYSIS

Data were analysed using SPSS version 23 statistical package to test for statistical significance.

RESULTS

Out of 433 combined samples of fresh faeces, cloacal and laryngeal swabs investigated, 26(6%) isolates were phenotypically identified as *E. coli* O157:H7 by characteristic appearance on CT-SMAC agar, confidence interval (0.46,0.55); (Table II). Of the 26 non-sorbitol fermenting (NSF) isolates, 73%(19) were isolated from cloacal swabs. Faeces and laryngeal swabs had 19.2 %(5) and 7.7%(2) isolates respectively; (Table III).

Serology

All 26 non-sorbitol fermenting (NSF) isolates were negative on Wellcolex* *Escherichia coli* O157:H7 kit R30959601 (Remel Europe Ltd, Darford Kent, UK).

Multiplex polymerase chain reaction (PCR)

All 26 isolates were amplified at 401bp confirming them primarily as *E. coli* and none was positive for *rfbEO*157 and *fliCh*7. Positive control *E. coli* O157:H7 (LMG 21756) was amplified to 327bp and 247bp respectively; (Plate 1).

Table II: Identification of presumptive *Escherichia coli* O157:H7 by sorbitol fermentation

Identification of Presumptive *E. coli* O157:H7			
Location (District)	**No. of Farms**	**No. of Samples**	**NSF(%)**
Du	5	103	3(2.9)
Zawan	4	92	6(6.5)
Kuru	4	76	3(3.9)
Vwang	3	80	8(10)
Gyel	4	82	6(7.3)
Total	20	433	26(6)

DISCUSSION

Attempts at isolation of *E. coli* O157:H7 in avian species have yielded varied results globally. Compared to cattle and sheep considered as natural reservoirs of this organism, limited information on characteristics and prevalence of shiga-toxin producing *E. coli* (STEC) in avian species is available (Nataro and Kaper, 1998). Reports of colonization of chicken cecae, and faecal shedding of *E. coli* O157:H7 suggested that chicken could serve as host or reservoir host for *E. coli* O157:H7 (Dipineto *et al*., 2006).

This study did not yield any confirmed isolate of *E. coli* O157:H7 in the area studied. All 26 non-sorbitol fermenting (NSF) *E. coli* isolates which were presumed positive phenotypically were negative by serology and PCR using Wellcolex* *Escherichia coli* O157:H7 kit R30959601 (Remel Europe Ltd, Dartford Kent, UK) and *E. coli* O157:H7 serotype specific genes

TABLE III: Distribution of Non-sorbitol fermenting *Escherichia coli* in relation to geographic location and source of sample

Location (District)	No. of Farms	Source of Samples				No.(%) of NSF *E. coli*			Total
		Faeca	Cloaca	Larynea	Total	Faeca	Cloaca	Laryngea	
Du	5	36	46	21	103	-	3(2.9)	-	3(2.9)
Zawan	4	33	41	18	92	1(1)	3(3.3)	2(2.2)	6(6.5)
Kuru	4	36	30	10	76	-	3(3.9)	-	3(3.9)
Vwang	3	50	30	0	80	3(3.8)	5(6.2)	-	8(10)
Gyel	4	50	32	0	82	1(1.2)	5(6.1)	-	6(7.3)

95%CI-Du 0.18, 0.26; Zawan 0.57, 0.66; Kuru 0.50, 0.59; Vwang 0.00, 0.00; Gyel 0.00, 0.00
NSF= non-sorbitol fermenting
95%CI= 95% Confidence interval

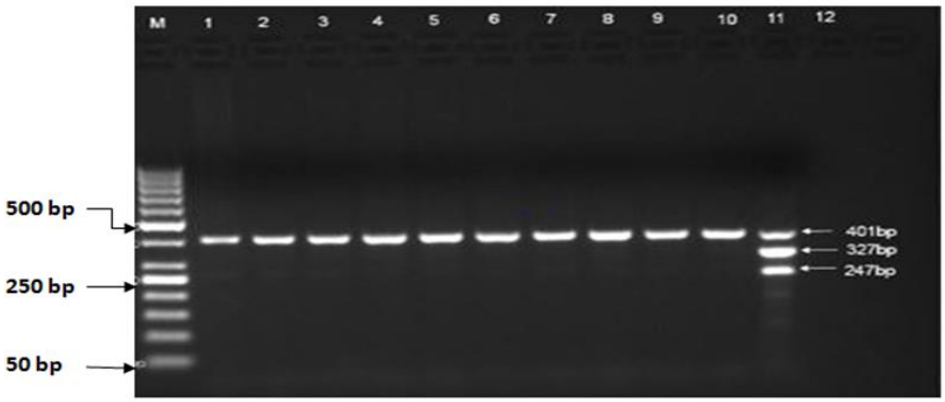

Plate 1: Detection of *rfbE0157* and *fliCh7* with *E16S rRNA* as internal control. Lane M 50bp DNA ladder (Fermenter®), lane 1-10 *E. coli* chicken isolates, lane 11 positive control; *Escherichia coli* (LMG 21756), lane 12 negative control (nuclease free water). All 26 isolates were negative for *rfbEO157* and *fliCh7*, control sample was amplified to the expected 327bp and 247bp for *rfbE0157* and *fliCh7* genes respectively. All 26 isolates including positive control were amplified at 401bp.

rfbEO157 and *fliCh7* (Plate 1). The contents of CT-SMAC media inhibits partially or completely growth of other *E. coli* strains (Zadik *et al*., 1993), hence most other strains were not expected to grow in such media. However, Park *et al*., 2011 reported false positive results on CT-SMAC medium from non-sorbitol fermenting bacteria such as *Hafnia alvei*, *Proteus spp*, *Providencia spp*, *Aeromonas spp* and *Morganella morganii*. In this study, all 26 NSF isolates were positive for *E16SrRNA* gene confirming all primarily as *E. coli*. Thus, the phenotypic similarities with *E. coli* O157:H7on CT-SMAC agar may suggest the presence of other *E. coli* serotypes other than O157 which shares phenotypic characteristics on CT-SMAC (March And Ratnam.,1986).

Related studies in other parts of the world yielded negative findings. In a one year study of 1000 faecal samples in the United Kingdom (UK) by Chapman *et al*., (1997) they were unable to find *E. coli* O157:H7. In the Czech Republic, Cizek *et al*., (1999) did not find *E. coli* O157:H7 in 50 pigeons and 20 sparrows respectively. Hajian *et al*., (2011) and Miri *et al*., (2014) reported no isolation of *E. coli* O157:H7 in 82 chicken meat samples and 70 chicken nugget samples in Iran. Although, in Ado-Ekiti, South West Nigeria; Oluyege and Famurewa (2015) reported non

isolation of verotoxigenic *E. coli E. coli* O157:H7 in intensively reared layer hens and broiler chickens, they found *E. coli* O157:H7 and non O157 in local chickens reared under free range systems which they probably acquired from the environment where ruminants are also kept and grazed.

In contrast to our findings, some positive results of isolation of *E. coli* O157:H7 have been reported by other researchers in poultry and other avian species. Kalin *et al*., (2012) had a 0.1% and 0.4% isolation of *E. coli* O157:H7 from liver and caecum samples respectively of 1000 chickens investigated in Turkey. All the isolates were positive for *eaeA* gene; a virulence factor responsible for human infections (Wang *et al*., 2002). Also in Turkey, Akkaya *et al*., (2006) isolated *E. coli* O157:H7 from 2 of 190 chicken carcass samples. The isolates were positive for *stx1* and *stx2*, an indication that poultry meat can also be a source of infection with *E. coli* O157:H7. Similarly, Dipineto *et al.*, (2006) reported 3.6% (26/720) isolation of *E. coli* O157:H7 in intensively managed living layer hens in Italy. The isolates were positive for *stx2*, *eaeA* and *hlyA* genes. This finding is significant because of the presence of the virulence factor enterolysin (*hlyA*) which often accompany human pathogenic strains (Dipineto *et al.*, 2002). Akbar *et al.*, (2014) reported 2% isolation of *E. coli* O157:H7 from 152 poultry meat samples in Pakistan. Wang *et al.*, (2014) reported isolation of 1.43% in 140 faecal samples and 1.67% in 60 chicken meat samples in Eastern China. In the UK, Wallace *et al*., (1996) isolated *E. coli* O157:H7 from faecal samples of gulls.

In Africa, Raji *et al*., (2006) reported a 9.6% isolation of *E. coli* O157:H7 in free range chicken raised integrated with goats and cattle in Morogoro, Tanzania. This high rate of isolation may be explained by the management system which allowed the chicken access to pastures and environment contaminated by goat and cattle faeces. Kagambega *et al.*, (2012) also reported 6% prevalence of STEC in chicken carcasses slaughtered in Burkina Fasso. In Nigeria, reports on isolation of *E. coli* O157:H7 in avian species is scanty. Aibinu *et al.*, (2007) reported 10% isolation from 50 faecal samples of chicken in Lagos using serological methods. This rate is high compared to 2.5% isolation in free ranged chicken by Oluyege and Famurewa (2015) in Ado-Ekiti, South West Nigeria. Elsewhere, Fuh *et al*., (2018) reported 6.67% and 1.94% isolation of *E. coli* O157:H7 from faeces in free-ranged and intensively reared chicken in Cross River; South-South Nigeria. The non-isolation of *E. coli* O157:H7 by Oluyege and Famurewa (2015) in intensively managed chicken is in agreement with our findings where all the 26 *E. coli* NSF isolates was negative for *E. coli* O157:H7 by serology and PCR. This result is suggestive that intensively managed chicken may not be carriers or reservoir host for *E. coli* O157:H7 in Jos Central Nigeria. Further research is required to support this finding. The variation in isolation rates observed globally could be attributed to the sensitivity of the methods of isolation. Methods involving enrichment with trypton soy broth (TSB), immunomagnetic separation (IMS) and hydrophobic grid membrane filter-immunoblot procedures and PCR produce a higher isolation rate (Wallace *et al*., 1997).

LIMITATIONS

This research was limited by funds. This reduced our ability to characterize the isolates for virulence genes and full identification of the isolates.

ACKNOWLEDGEMENT

The management and staff of National Veterinary Research Institute, Vom: particularly, Staff of Central Diagnostics and Biotechnology Departments are most appreciated for providing research facilities and for their criticisms and assistance. We also thank Dr. Emmanuel Obishakin for his assistance and for the control strain *E. coli* O157:H7 (LMG 21756) sourced

from Belgium and Fatima Liman and Dinchi Davou for their patience and sacrifices.

REFERENCES

AIBINU, I.E., PETERS, F.R., AMISU, K.O., ADESIDA, S.A., OJO, M.O. and TOLU, O. (2007). Multidrug Resistance in *E.coli* O157 Strains and the Public Health Implication. *Journal of American Science*; 3(3): 22-33.

AKBAR, A., SITARA, U., KHAN, S.A., ALI, I., KHAN, M.I., PHADUNGCHOB, T. and ANAL, A.K. (2014). Presence of *Escherichia coli* in poultry meat: A potential food safety threat. *International Food Research Journal;* 21(3): 941-945.

AKKAYA, L., ATABAY, H.I., KENAR, B, and ALISARI, M. (2006). Prevalence of Verocytotoxigenic *Escherichia coli* O157:H7 on Chicken Carcasses Sold in Turkey. *Bulletin of the Veterinary Institue in Pulawy;* 50: 513-516.

ALBARRI, O., VAR, I., MERAL, M., Bedir, b and FATIHKOKSAL, B.H. (2017). Prevalence of Escherichia coli isolated from meat, chicken and vegetable samples in Turkey. *Journal of Biotechnology Science Research*; Vol. 4(3): 214-2022.

CHALMERS, R.M., AIRD, H. and BOLTON, F.J. (2000). Waterborne *Escherichia coli* O157.
Symposium series Society for Applied Microbiology; 29:124S-132S.

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 of Infectious Diseases*; 119:245–250.

CIZEK, A., ALEXA., P.., LITERAK., I., HAMRIK, J., NOVAK, P. and SAMOLA, J. (1999). Shiga toxin-producing *Escherichia coli* O157 in feedlot cattle and Norwegian rats from a large-scale farm.*Letters in Microbiology*; 28: 435-439.

DIPINETO, L., SANTANIELLO, A., FONTENELLA, M., LAGOS, K., FIORETTI, A. and MENNA, L. F. (2006). Presence of Shiga toxin-producing *Escherichia coli* O157:H7 in living layer hens. *Letters in Applied Microbiology*; 43:293–295.

DOYLE, M.E., ARCHER, J., KASPAR, C.W. and WEISS, R. (2006). Human Illness Caused by *Escherichia coli* O157:H7 from Food and Non-Food Sources. FRI Briefings.CDC. *Escherichi* O157:H7.

DOYLE, M.P. and SCHOENI J.L., (1987). Isolation of Escherichia coli O157:H7 from retail fresh meats and poultry. *Applied Environmental Microbiology;* 53:2394–2396.

FUH, N.J., CHRISTIANA, O.M., YAMI, A.L., UTEH, U.P., EKPIKEN, E.S. and OGECHI, U.M. (2018). Prevalence and Antibiotic Resistance of Escherichia coli O157:H7 serotype from Chicken Droppings Produced by Free-Ranged and Poultry Birds in Cross River, Nigeria. *American Journal of Biomedical and Life Sciences.* 6(3): 51-55

HAJIAN, S., RAHIMI, E. and MOMMTAZ, H. (2011). A 3-year study of *Escherichia coli* O157:H7 in cattle, camel, sheep, goat, chicken and beef minced meat. *International Conference on Food Engineering Biotechnology* .

HAMZA, A.M, HUSSEIN, A.M, and KHALEF, J.M, (2013): Isolation of *Escherichia coli* O157:H7 Strain from Faecal Samples of Zoo Animal. *The Scientific Journal;* 843968.

ISIBOR, J.O., EKUNDAYO, A.O., OHENHEN, R.E. and ORHUE, P.O. (2013). *Escherichia coli* O157:H7- Prevalence and Risk Factors of Infection in Edo State, Nigeria. *American Journal of Research Communicatio;* 1(3): 35-50.

KAGAMBEGA, A., MARKITAINEN, O., SIITONEN, A., TRAORE., A.S., BARRO, N. and HAUKKA, K., (2012). Prevalence of diarrhoeagenic *Escherichia coli* virulence genes in the faeces of slaughtered cattle, chickens, and pigs in Burkinafaso. *Microbiology Open*; 1:3:276-284.

KALIN, R., ONGOR, H. and CETINKAYA, B. (2012). Isolation and Molecular Characterization of *Escherichia coli* O157 from Broiler and Human Samples. *Foodborne Pathogens and Disease*; 9:4:313-318.

MARCH, S. B., and RATNAM. S. (1986). Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. *Journal of Clinical Microbiology*; 23:869–872.

MEAD, P.S. and GRIFFIN, P.M. (1998). *Escherichia coli* O157:H7. The Lancet; 352:9135:1207- 1212.

MICHINO, H., ARAKI, K., MINAMI, S., TAKAYA, S., SAKAI, N., MIYAZAKI, M., ONO, A. and YANAGAWA, H. (1999). Massive Outbreak of *Escherichia coli* O157:H7 Infection in Schoolchildren in Sakai City, Japan, Associated with Consumption of White Raddish Sprouts. *American Journal of Epidemiology*; 150(8):787-796.

MIRI, A., RAHIMI, E., MIRLOHI, M., MAHAKI, B.,JALALI, M and SAFAEI, H.G. (2014). Isolation of Shiga toxin-producing Escherichia coli O157:H7 from hamburger and chicken nugget. *International Journal of Environmental Health England. 3(20).*

NATARO, J.P and KAPER, J.B. (1998). Diarrheagenic Escherichia coli. *Clinical Microbiology Reviews*; 11(1): 142-201.

OLATOYE, I.O., AMOSUN, E.A., and OGUNDIPE, G.A. (2012). Multidrug Resistant *Escherichia coli* O157 Contamination of Beef and Chicken in Municipal Abattoirs of South West Nigeria. *Nature and Science;* 10(8): 125-132.

OLUYEGE, A.O., and FAMUREWA, O. (2015). Shiga Toxin and Non-Shiga Toxin-Producing *Escherichia coli* O157 from Cattle, Goats and Chicken in Ado-Ekiti, South West, Nigeria. *International Journal of Tropical Disease and Health*; 6(4): 108-118.

PARK, S-H., RYU, S AND KANG, D-H. (2011). Improved Selective and Differential Medium for Isolation of Escherichia coli O157:H7. *Journal of Clinical Microbiology* 49(1): 405-408

RAJI, M.A., MINGA, U.M., and MACHANG'U, R.S. (2006b). Prevalence and Characterisation of Verotoxigenic Escherichia coli O157 *Isolated* from Local Chicken in Morogoro, Tanzania. *Journal of Animal and Veterinary Advances* 5 (11): 952-958, 2006.

REUBEN, C.R. and MAKUT, M.D. (2014). Occurrence of Escherichia coli O157:H7 in vegetables grown and sold in Lafia metropolis, Nigeria. *World Journal of Microbiology*;1(3):017-021.

RILEY, L. W., REMIS, R. S., HELGERSON, S. D., MCGEE, H. B., WELLS, J. G., DAVIS, B. R., HEBERT, R. J., OLCOTT, E. S., JOHNSON, L. M., HARGRETT, N. T., BLAKE, P. A., and COHEN, M. L. (1983): Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *The New England Journal of Medicine;* 308**:**681-685.

RWEGO, I.B., GILLESPIE, T.R., ISABIRYA-BASUTA, G. and GOLBERG, T.L. (2008). High rates of *Escherichia coli* Trasmission between Livestock and Humans in Rural Uganda. *Journal of Clinical Microbiology*; 46 (10): 3187-3191.

WALLACE, J.S., CHEASTY, T. and JONES, K. (1997). Isolation of Vero cytotoxin-producing *Escherichia coli* O157 from wild birds. *Journal of Applied Microbiology*; 82: 399-404.

WANG, G., CLARK, C.G. and RODGERS, F.G. (2002). Detection in *Escherichia coli* of Genes Encoding the Major Virulence Factors, the Genes Defining the O157:H7 Serotype, and Components of the Type 2 Shiga Toxin Family by Multiplex PCR, *Journal of Clinical Microbiology*, 40(10): 3613-3619.

WANG, S, ZHANG, S, LIU, Z, LIU, P, SHI, Z, WEI, J, SHAO, D, LI, B, and MA, Z, (2014): Molecular Characterization of Enterohemorrhagic *E. coli* O157 Isolated from Animal Faecal and Food Samples in Eastern China. *The Scientific World Journal;* Vol. 2014.

WANI, S.A., SAMANTA, I., BHAT, M.A. and NISHIKAWA, Y.(2004). Investigation of shiga toxin-producing *Escherichia coli* in avian species in India. *Letters in Applied Microbiology;* 39,389-394.

WITOLD, A. F. and CAROLYN, J. H. (2011). *Escherichia coli* O157:H7: Animal reservoir and sources of infection. *Foodborne Pathogens and Diseases*; 8:4: 465-487.

WETZEL, A.N. and LEJEUNE, J.T. (2007). "Isolation of *Escherichia coli* O157:H7 strains that do not produce Shiga toxin from bovine, avian and environmental sources," *Letters in Applied Microbiology*; 45: 504–507.

ZADIK, P. M., CHAPMAN, P. A. and SIDDONS, C. A. **(**1993). Use of tellurite for the selection of verocytotoxigenic *Escherichia coli* O157. *Journal of Medical Microbiology.* 39:155–158.