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Molecular Detection of Virulence Genes of Escherichia Coli O157 Isolated from Different Sources

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ABSTRACT

Escherichia coli O157 is recognized as an important foodborne pathogen responsible for sporadic cases to serious outbreaks worldwide. The morbidity and mortality associated with several recent outbreaks due to STEC have highlighted the threat this organism possess to public health. Bacteriological examination of 671 samples collected from cattle, sheep, chickens, ducks, milk and water showed that 34.3% (230/671) were positive E. coli. Out of 230 isolates of positive E. coli, (16) isolate were identified by vitek2 system as E. coli O157 (6.9%) with high incidence in cattle samples (20%) then chicken and water samples (13.6%) and (12.5%) respectively. On the other hand, there were no isolates from ducks samples for E. coli O157 and with low percent (7.8%) from raw milk samples and (1%) from sheep samples. The application of PCR for screening of virulence genes (sxt1, sxt2 and eaeA) in E. coli O157 isolates revealed that out of 16 isolates of E. coli O157 there were (9), (6) and (16) were positive for stx1, stx2 and eaeA, respectively. This study concluded that molecular identification by PCR for detection of E. coli O157 virulence genes is rapid, specific and accurate approach in studying the actual role of these genes in disease pathogenicity.

1. INTRODUCTION

Escherichia coli is a member of the family Enterobacteriaceae, facultative anaerobic, Gram-negative short rods and considered a common inhabitant of the gut of warm-blooded animals, including man, but also be found in water, soil, or other environments because of fecal contamination. WHO (2016). The enteric *E. coli* is classified on the basis of virulence properties into enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC), verotoxigenic (VTEC) or enterohemorrhagic (EHEC), and enteroaggregative *E. coli* (EAggEC). Hammerum and Heuer, (2009). *Escherichia coli* O157 is generally identified as being a Non-Sorbitole Fermenting, (NSF) Gram negative rod shaped organism, ranging 0.7 to 1.5 x 2 to 5 µm in size, oxidase negative, catalase positive and indole positive. Some of these characteristics are used for biochemical confirmation of *Escherichia coli* O157 Heba (2012).In human, *Escherichia coli* O157is an important emerging zoonotic foodborne pathogen that especially in infants and the elderly can cause watery and/or bloody diarrhea, hemorrhagic colitis (HC), hemolytic-uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP) Gyles (2007) and Xiong *et al* ., (2012). The major animal reservoirs of EHEC O157 are primarily cattle, followed by sheep, goats, pigs, and chickens. Ferens *et al.*, *(*2011) and Yan, *et al.*, (2011). The resistance of powerful virulence factors to acid is mainly associated with the pathogenicity of *E. coli* O157. Shiga toxin and intimin, are key virulence factors for the pathogenesis of *E. coli* O157 Wu, *et al*., (2011). Intimin is encoded by

eae gene in the locus of enterocyte effacement (LEE) of *E. coli* O157 is essential for Attaching/Effacing (A/E) lesion formation. Shiga toxins are responsible for binding to the glycolipid globotriaosylceramide (Gb3) on the target cell surface causing protein synthesis to cease leading to the death of affected cells Kaper et *al*., (2004). This study was conducted to identify *Escherichia coli* O157 by VITEK2 system, prevalence of important virulent genes (stx1, stx2 and eaeA) of *Escherichia coli* O157 isolated from different sources.

2- MATERIALS AND METHODS

2.1. Sample collection

A total of 671 samples were collected from different sources in Egypt. Sampling included various types of water, raw milk, fecal swabs from diarrheic animal cases and birds cloacal swabs.

2.2. Isolation of *E. coli* O157

Twenty five ml of each sample of water and milk was enriched with 225 ml of modified tryptic soy broth (mTSB- DifcoLa Jolla, CA/USA) and incubated for 24 h at 37°C. Also each fecal swab was directly transferred to 10 ml enrichment broth as described by **Karuniawati (2001).** After enrichment, aliquots of 100 µl were plated onto MacConkey s agar (MAC-Difco) and Eosin Methylene Blue (EMB-Difco) agar and onto Sorbitol MacConkey s agar (SMAC-Difco) to test for sorbitol non-fermenting bacteria (colorless colonies). After 18 to 24 h at 37°C, characteristic colonies from SMAC agar were transferred onto Tryptic Soy agar (TSA, Difco) and used for biochemical identification tests.

2.3. Identification and biochemical characterization of isolates

Purified suspected *E. coli*-like colonies (n = 230) were identified by examining the morphology and biochemical properties of growing colonies. Gram staining was evaluated following the procedure described by **Cruickshank *et al*., (1975)** and *E. coli*-like colonies were subjected to different biochemical tests, including sugar fermentation tests, indole production test, Methyl-Red and Voges- Proskauer (IMIVC) tests, following the standard methods described by **Holt *et al*., (1994) and Quinn *et al*., (2002)**.

2.4. E. coli O157 identification by Vitek2 compact system according to BioMe'rieux (2006)

Inoculum Preparation: From the isolated colonies grown on the Mueller Hinton agar 24 h at 37°C, a bacterial suspension was prepared in 3 mL of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a 12x75 mm clear plastic (polystyrene) test tube. The turbidity of the suspension was adjusted to a McFarland standard of 0.5 with the help of a VITEK-2 DensiCheck instrument. The time between the preparation of inoculum and filling of the card was always less than 30 min.

Identification with the VITEK-2 system was performed using a Gram Negative (GN) card according to the Manufacturer's instructions. The culture suspension was inoculated into the GN Card with the help of a vacuum device inside the filling chamber. The cards were later transferred into the loading chamber where the cards were sealed and were incubated in a rotating carousel at 37°C. Each loaded card was removed from the carousel for every 15 minutes, transported to the optical system for reaction readings and the returned to the carousel incubator until the next read time. Data was collected at 15-minute intervals during the entire incubation period.

2.5. Serotyping identification of *E. coliO157* isolates:

All ***E. coli*** isolates were serotyped by slide agglutination test according to **Edwards and Ewings (1972)** using standard monovalent ***E. coli* O157** antisera.

2.6. Congo red (CR) binding activity

The individual *E. coli* O157 isolates were tested for their binding activity with Congo red dye, which is an indicator of intestinal invasion **Berkhoff and Vinal (1986)**. Individual *E. coli* O157 colonies were cultured onto Congo red medium and incubated at 37°C for 24 h. Culture plates were then transferred at room temperature for additional 24-48h of incubation. Growth of red colonies indicates a Congo red positive (CR+).

2.7. Detection of virulence genes

2.7.1. DNA extraction. DNA extraction from isolates was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^{O}C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primer. Primers used were supplied from **Metabion (Germany)** are listed in **table (1).**

2.7.2. PCR amplification. For eaeA gene PCR, primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. However in **stx1 and stx2 PCR,** primers were utilized in a 50- µl reaction containing 25 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 9 µl of water, and 12 µl of DNA

template. The reactions were performed in an Applied biosystem 2720 thermal cycler.

2.7.3. Analysis of the PCR Products: Sambrook, *et al.*, (1989)

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the products was loaded in each gel slot. Gelpilot 100 bp and 100 bp plus DNA Ladders (Qiagen, Germany, GmbH) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table (1): Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences		Amplified segment (bp)	Primary Denaturation	Amplification (35 cycles)			Final extension	Reference
					Secondary denaturation	Annealing	Extension		
Stx1	F	ACACTGGATGATCTCAG TGG	614	94˚C 10 min.	94˚C 1 min.	58˚C 1 min.	72˚C 1 min.	72˚C 10 min.	**Dipineto *et al.*, 2006**
	R	CTGAATCCCCCTCCATTA TG							
Stx2	F	CCATGACAACGGACAGC AGTT	779						
	R	CCTGTCAACTGAGCAGC ACTTTG							
eaeA	F	ATG CTT AGT GCT GGT TTA GG	248	94˚C 5 min.	94˚C 30 sec.	51˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	**Bisi-Johnson *et al.*, 2011**
	R	GCC TTC ATC ATT TCG CTT TC							

3- RESULTS AND DISCUSSION

Escherichia coli O157 is a serotype of the bacterial species *Escherichia coli* and is one of the Shiga toxin–producing types of *E. coli*. It is a cause of disease, typically foodborne illness through consumption of contaminated and raw food, including raw milk Karch *et al*., (2005). Infection with this type of pathogenic bacteria may lead to hemorrhagic diarrhea, and to kidney failure Tamparo and Carol (2011), these have been reported to cause the deaths of children younger than five years of age, of elderly patients, and of compromised patients. Transmission is via the fecal–oral route, and developed illness has been through distribution of contaminated raw leaf green vegetables, undercooked meat and raw milk CDC (2016). *Escherichia coli* O157 causing severe bloody or mucoid diarrhea in calves and some of these animals died in sever complicated cases. A recent outbreak of fatal meningoencephalitis and septicemia in one-month-old goats was caused by Verotoxogenic *Escherichia coli* O157 (VTEC O157) had been reported by CFSPH (2016).

Verotoxogenic *Escherichia coli* O157 considered one of the causes of the avian colibacillosis, which considered as one of the principal causes of heavy economic losses to the poultry industry. Avian colibacillosis has been noticed to be a major infectious disease in birds of all ages, but very dangerous to chicks Kabir (2010). The technique of vitek2 system has improved the field of bacterial screening by providing more reliable, faster and highly sensitive technique for bacterial identification. In addition of identification of bacteria by VITEK2 system has revealed prominent inter laboratory reproducibility and is quickly being included as a routine method for laboratory microbiology Wallet *et al.*, (2005). A total of 16 *E. coli* O157 isolates were recovered from 671 samples collected from different sources and identified by vitek2 system. Table (2) showed that the prevalence of *E. coli* O157 from cattle fecal samples was (20%).

The prevalence of VTEC O157 from cattle feces ranged from (0.2%) to (27.8%) Hussein and Bollinger, (2005). Cernicchiaro et *al*., (2012) reported (19%) bovine fecal samples were positive for *E. coli* O157. The prevailing part of the *E. coli* O157 from sheep fecal samples was (1%). The prevalence of *E. coli* O157 in sheep was (1.4%) Novotna et *al*., (2005). In Scotland a higher incidence of *E. coli* O157 among sheep fecal samples was (6.5%) Ogden *et al*., (2005). *E. coli* O157 present among (0.7%) of sheep in Great Britain Milnes, (2008). Where the *E. coli* O157 from chickens cloacl swabs was (13. 6%). Abdul-Raouf *et al*., (1996) recorded low incidence of O157 in chicken (4%). While almost similar incidence (11%) from chicken was reported by Heba (2012). The *E. coli* O157 from milk samples was (7.8%). These percentages was near to those obtained by other authors as Mohamed *et al.,* (2003) who reported (7.1%) positive for *E. coli* O157 were from raw milk samples. Also Abdul-Raouf *et al*., (1996) obtained that, *E. coli* O157 in raw milk samples was (6%). The prevalence of the *E. coli* O157 from fresh water were (12.5%).

Similar result (12.5%) positive for *E. coli O157* from Nile river samples was obtained by Mohamed et al., (2003). However Mohmmed, et al., (2012) reported a higher incidence (23%) were positive for *E. coli* O157 isolates from drinking water in Basrah Province.

Table (2): Prevalence, virulence factors and genes among *E. coli* O157 isolates:

Source	Type of Samples	Isolated *E.coli*	Positive *E.coli* O157		Invasion of *E.coli* O157 on Congo Red Agar	Virulence genes		
			+VE	%		Stx1	Stx2	eaeA
Cattle	Fecal swabs	30	6	20	+++	5	4	6
Sheep	Fecal swabs	100	1	1	+++	1	0	1
Chicken	Cloacal swabs	22	3	13.6	+++	2	1	3
Duck	Cloacal swabs	20	0	0		0	0	0
Milk	Raw milk	38	3	7.8	+++	0	0	3
Water	Animal drinking water	24	3	12.5	+++	1	1	3

On other hand, there were no isolates for *E. coli* O157 from ducks cloacal swabs were detected. Similar result was recorded by Heba (2012) who found no isolates from ducks for *E.coli* O157. But Shaohui *et al*., (2014) reported that, 1% was positive for *E. coli* O157 from duck samples. As explained in (Table 2), all *E. coli* O157 isolates clearly demonstrated an invasive phenotype on CR agar, similar findings were reported by Samy *et al*., (2013) and Shome *et al.*, (2005).The pathogenesis of *E. coli* O157 were established by Shiga toxins and intimin genes. The Intimin is encoded by eaeA gene is essential for Attaching/Effacing (A/E) lesion formation this lesion is characterized by the destruction of the microvilli and the rearrangement of the cytoskeleton to form a pedestal-like structure that cups the bacteria individually for colonization of the gastrointestinal tract Woodward *et al*., (2003).

Shiga toxins which consists of five identical B subunits that are responsible for binding the holotoxin to the glycolipid globotriaosylceramide (Gb3) on the target cell surface, and a single A subunit that cleaves ribosomal RNA, causing protein synthesis to cease leading to the death of affected cells Newton *et al*. (2009). Out of the 16 isolates of *E. coli* O157 virulence genes 9 (56.25%), 7(43.75%) and 16(100%) were positive screened for stx1, stx2 & eaeA, respectively as shown in figure (1, 2, 3, 4). These were near to those obtained by Khanjar, et *al.*, (2014). The distribution and role of these studied genes in pathogenicity of E.coli were previously investigated in several studies researches as reported by Shahzad et al., (2013) who concluded that *Stx1* and *Stx2* genes were found in 38% of E.coli isolates of raw milk and beef meat contaminated with feces of animals. Moreover, Adler *et al.*, (2000) revealed the distribution of sxt1 and sxt2 in beef and milk samples were 1.4 and 41.2% respectively, and both of them detected in 57.4%.

According to the results of Barkocy-Gallagher *et al.*, (2001) suggested that most of SNF *E. coli of* bovine isolates carried the both the shiga toxin genes (sxt1 and sxt2). However, the prevalence rate of sxt2 (20%) is more predominant than *Stx1* gene 3%, and both the genes detected in 76 % isolates of human clinical cases Stephen *et al.*, (1989). The role of eaeA gene that encodes intimin is considered critical in the attaching and colonization of the organism into the host epithelial cells that usually lead to hemolytic uremic syndrome in human James et al., (2001).

This also supported by the findings of Oporto et al., (2008) that demonstrated , all *E. coli* O157:H7 carried *stx*(2) gene and *eaeA gene* was detected in 95.9% and the distribution of stx(2)/eaeA/E-hlyA genes were detected in 75.5% of O157:H7 isolates, although the non-O157 STEC, contain the eaeA gene but with significantly lower percentage (5.3%) with sporadic association of E-hlyA gene in 50.2% of the isolates.

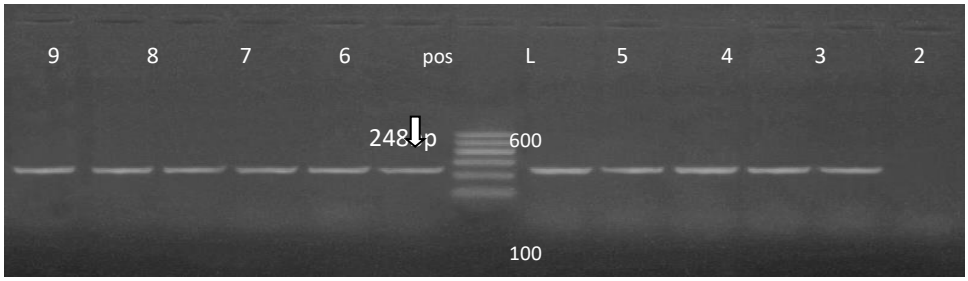

Fig.(1): Agarose gel electrophoresis showing results of uniplex PCR for detection of *eaeA* gene. from samples No. (1 to 10) of *E.coli* O157. (Lane.1to lane.6):Positive *eaeA* gene (248 bp)from cattle samples. Lane 7: Positive of *eaeA* gene (248 bp) from sheep samples . (lane.8 to lane. 10): Positive of *eaeA* gene (248 bp) from chicken samples. Neg : Negative control. L: represents the molecular size marker (100pb plus ladder) Pos : Positive control of *eaeA* gene (248 bp).

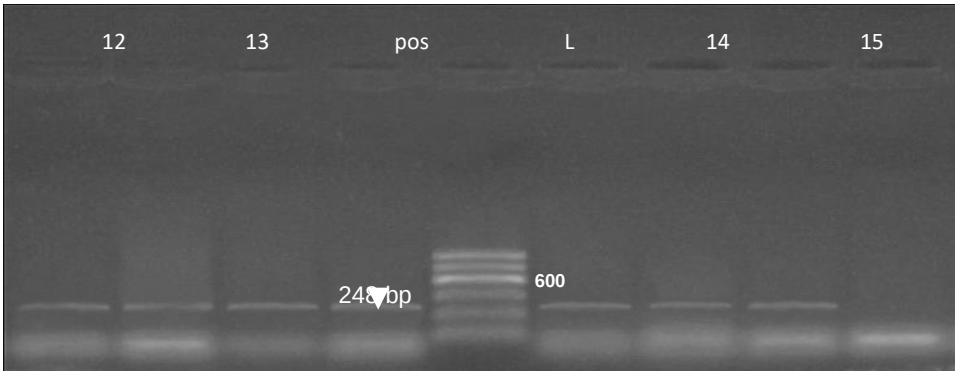

Fig. 2: Agarose gel electrophoresis showing the continuous results of uniplex PCR for detection of *eaeA* gene from samples No. (11 to 16) of ***E. coli*** O157. Lane.11 to lane.13: Positive ***eaeA* gene** (248 bp) from milk samples. Lane.14 to lane.16: Positive ***eaeA* gene** (248 bp) from water samples. L: represents the molecular size marker (100pb plus ladder). Neg : Negative control . Pos : Positive control of ***eaeA* gene** (248 bp).

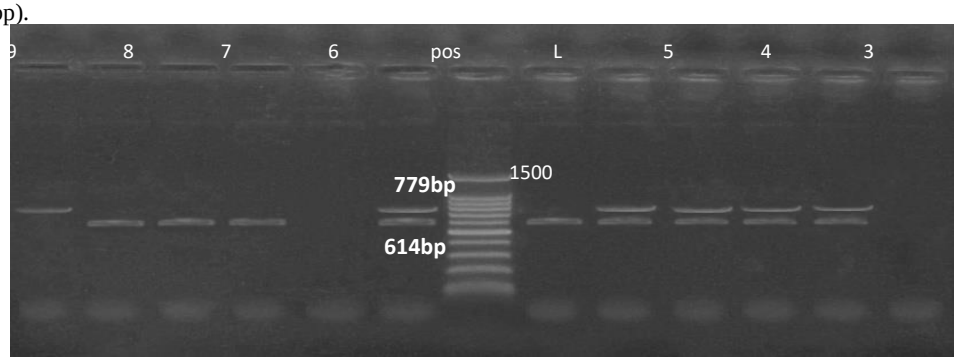

Fig.3 : Agarose gel electrophoresis showing results of uniplex PCR for detection of *(stx1 and stx2)* genes. from samples No. (1 to 10) of *E.coli* O157. (Lane.1to lane.6): Positive stx1 (614 bp) and stx2 (779 bp) gene from cattle samples. Lane 7: Positive of stx1 (614 bp) and stx2 (779 bp) gene from sheep samples. (lane.8 to lane. 10): Positive of stx1 (614 bp) and stx2 (779 bp) gene from chicken samples. Neg : Negative control . L: represents the molecular size marker (100pb plus ladder) Pos :Positive control of stx1 (614 bp) and stx2 (779 bp) gene.

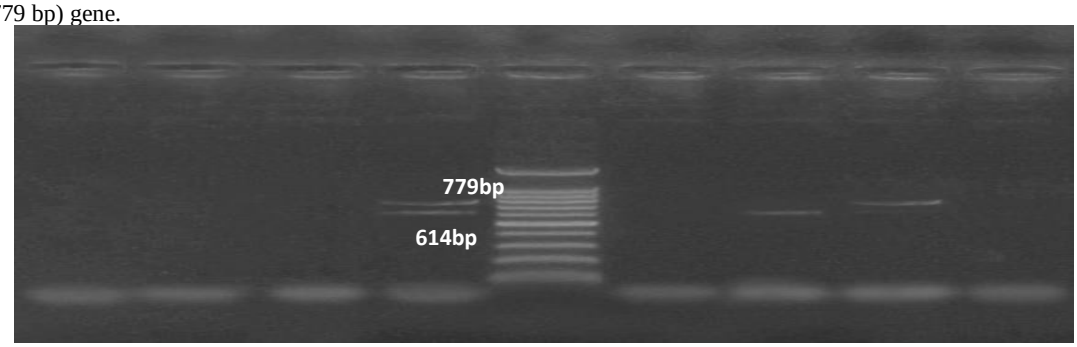

Fig.(4): Agarose gel electrophoresis showing the continuous results of uniplex PCR for detection of *stx1 and stx2* genes from samples No. (11 to 16) of *E. coli* O157. Lane.11 to lane.13: Positive stx1 (614 bp) and stx2 (779 bp) gene. from milk samples. Lane.14 to lane.16: Positive stx1 (614 bp) and stx2 (779 bp) gene from water samples. L: represents the molecular size marker (100pb plus ladder). Neg : Negative control . Pos : Positive control of stx1 (614 bp) and stx2 (779 bp) gene.

5- CONCLUSIONS

Animal and bird feces, particularly from cattle, sheep and chicken, serve as major reservoirs for water contamination by *E. coli* O157. Thus, it is important in building up control methods for

prevention of contamination by *E. coli* O157 in animals and humans. Several virulence factors associated with *E. coli* O157 pathogenicity were

investigated. The results in our study concluded that molecular identification by PCR was accurate, rapid and helpful approach in detection of E.coli O157 virulence genes isolated from different sources. The Intimin gene was detected in all tested isolates while stx1, stx2 were detected in 56.25% and 37.5% of samples respectively. Further researches about the actual role and distribution of these genes from different sources are needed in the future.

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6- REFERENCES

Abdul-Raouf, U. M., Ammar, M. S., Beuchat, L. R. 1996.Isolation of Escherichia coliO157:H7 from some Egyptian foods. Int. J. Food Microbiol. 29 (3): 423-426.

Adler, R.O., Keen, J. E., Siragusa, G.R., Barkocy-Gallagher, G.A., Koohmaraie, M., Laegreid, W.W. (2000): Correlation of enterohemorrhagic Escherichia coli O157 prevalence in feces, hides, carcases of beef cattle during processing. Proc. Natl. Acad. Sci., USA, 97: 2999-3003.

Barkocy-Gallagher, G.A., Arthur, T.M., Siragusa, G.R., Keen, J.E.; Edler, R.O.; Laegreid, W.W., Koohmaraie, M. 2001. Genotypic analysis of Escherichia coli O157:H7 and O157 nonmotile isolates recovered from beef cattle and carcasses at processing plants in the Mid-western states of United States. App. Environ. Microbiol. 67: 3810- 3818.

Berkhoff, H. A., Vinal, A. C. 1986. Congo red medium to distinguish between invasive and noninvasive E. coli pathogenic to poultry.Avian Dis.30 (1):117 – 121.

Biomeriux VITEK-2 Compact ref Manual – Ref-414532. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard M7 A5. Normal committee for clinical laboratory standards. Wayne, Pa. 2000.

Bisi-Johnson, M.A., Obi, C. L., Vasaikar, S. D., Baba, K. A.,Hattori, T. 2011. Molecular basis of virulence in clinical isolates of Escherichia coli and Salmonella species from a tertiary hospital in the Eastern Cape, South Africa. Gut Pathogens.3 (1):9.

Centers of disease control and prevention (2016): "Reports of Selected E. coli Outbreak Investigations". CDC.gov.

Cernicchiaro, N., Pearl, D., McEwen, S., Harpster, L., Homan, H., Linz, G., Lejeune, J. 2012. Association of wild bird density and farm management factors with the prevalence of E. coli O157 in dairy herds in Ohio (2007-2009). Zoonoses Public Health. 59 (5): 320-329.

Cruickshank, H., Duguid, J. P., Marmoin, B. P., Swain, R.H.A. 1975. Medical Microbiology. The practice of medical microbiology, vol. 195, 12th Ed. Churchill Livingstone, Edinburgh. London and NewYork.

Dipineto, L.,Santaniello, A.,Fontanella, M., Lagos, K., Fioretti, A., Menna, L.F. 2006. Presence of Shiga toxin-producing Escherichia coli O157:H7 in living layer hens. Letters in Appl. Microbiol.43 (9): 293–295.

Edwards, P. R., Ewing, W. H. 1972. Identification of Enterobacteriacae. Minneapolis, Burgess Publishing Co., PP.709. Burgess Publishing Cp. Atlanta USA 3rd Ed.

Ferens, W. A., Hovde, C. J. 2011. Escherichia coli O157:H7: animal reservoir and sources of human infection. Foodborne Pathogens and Disease.8 (4): 465–478

Gyles, C. L. 2007. Shiga toxin-producing Escherichia coli. J. Anim. Sci. 85. (11):45–62.

Hammerum, A. M., Heuer, O. E. 2009. Human Health Hazards from Antimicrobial-Resistant Escherichia coli of Animal Origin. Clin. Infect. Dis.48 (4):916–921.

Heba, B. M. 2012. Phenotypic and genotypic study of Escherichia coli O157among the native and imported chickens, ducks and turkeys. Thesis for PHD degree.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T., Williams, S. T. 1994. Begay's Manual of DeterminativeBacteriology,9th ed. Williams and Wilkins, Hagerstown, MD.

Hussein, H. S., Bollinger, L. M. 2005. Prevalence of Shiga toxin-producing Escherichia coli in beef cattle. J. Food Prot. 68 (10): 2224-2241.

James, C.S., Stanley, K.N., Allison, H.E., Flint, H. J., Stewart, C.S., Sharp, R.J., Saunders, J.R., Maccarthy, A.J. 2001. Lytic and lysogenic infection of diverse E. coli and Shigella strains with a verocytotoxigenic bacteriophage. Appl. Environ. Microbiol. 67: 4335-4337.

Kaper, J.B., Nataro, J.P., Mobley, H.L. 2004. Pathogenic Escherichia coli. Nat. Rev. Microbiol. 2:123–140.

Karch, H., Tarr, P., Bielaszewska, M. 2005. Enterohaemorrhagic Escherichia coli in human medicine. Int. J. Med. Microbiol. 295 :(6–7): 405 -18.

Karuniawati, A. 2001. Untersuchungen von Umweltproben auf "viable but non culturable" Salmonellen and enterohemorrhagische Escherichia coli (EHEC). Doctor thesis, Hohenheim University, Faculty of Agriculture II, Germany.

Khajar, S., Adamu, M.T., Shamsul, B.M.T., Desa, M.N. 2014. A review on Escherichia coli O157:H7-The Super Pathogen. Health and the Environ. J. 5 (2): 78-93.

Lutful –Ka bir, S.M. 2010.Avian Colibacillosis and Salmonellosis: A Closer Look at Epidemiology, Pathogenesis, Diagnosis, Control and Public Health Concerns. Int. J. Environ. Res. Public Health 7:89-114.

Milnes, A.S., Stewart, I., Clifton-Hadley, F.A. 2008. Intestinal carriage of verocytotoxigenic Escherichia coli O157, Salmonella, thermophilic Campylobacter and Yersinia enterocolitica, in cattle, sheep and pigs at slaughter in Great Britain during 2003. Epidemiol Infect 136 (6): 739–751.

Mohamed, M. E. M., Mohamed, A. A., Gharib, A. A. 2003. Zoonotic and microbiological aspects of Escherichia coli O157 with special reference to its RAPD-PCR. Vet. Med. J. Giza. 51(4):467-484.

Mohmmed, H. K.,Awatif, H. I., Farooq L. Jassim 2012. Detection of rfbO157 and fliCH7 Genes in Escherichia coli Isolated from Human and Sheep in Basrah Province.Raf. J. Sci. 23(1):19-33.

Novotna, R., Alexa, P., Hamrik, J., Madanat, A., Smola, J., Cizek, J. 2005. Isolation and characterization of shiga toxin producing Escherechia coli from sheep and goats from Jordan with evidence of multiresistant serotype O157:H7. Vet. Med. Czech. 50(3): 111-118.

Oporto, B., Esteban, J.I., Aduriz, G., Juste, R.A., Hurtado, A. 2008. Escherichia coli O157:H7 and non-O157 shiga toxin-producing E. coli in healthy cattle, sheep and swine herds in Northern Spain Zoon. Pub. Hlth. 55: 73-81.

Quinn, P.J., Carter, M.E., Markey, B.K., Donnoly, W.J., Leonard, F.C.2002. Veterinary microbiology and microbial disease.166-1117 Osney Mead, Oxford first LTd, Registered at the United Kingdom.

Sambrook, J., Fritscgh, E.F., Mentiates 1989. Molecular cloning. A laboratory manual. Vol1, Cold spring Harbor Laboratotry press, New York.

Samy, A. S., Salwa, F. A., Mohamed, H. A., Amira, M. Z., John, D. K., Domenico, P. 2013. Prevalence and characterization of shiga-toxin O157:H7 and non-O157:H7 EHEC isolated from different sources Afr. J. Microbiol. Res.7 (5):2637-2645.

Shahzad, K.A., Muhammad, K., Sheikh A.A., Yaqub, T., Rabbani, M., Hussain, T., Anjum, A.A., Anees, M. 2013. Isolation and molecular characterization of shiga toxin producing e. coli o157. J. Anim. Plant Sci. 23(6): 1618-1621.

Shaohui, W., Shuxiao, Z., Zhe, L., Pingping, L., Zixue, S., Jianchao, W., Donghua, S., Beibei, L., Zhiyong, M. 2014. Molecular Characterization of Enterohemorrhagic E. coli O157 Isolated from Animal Fecal and Food Samples in Eastern China. Sci. World J. (6):1-7.

Shome, B. R., Shome, R., Das, A., Mazumder, Y., Rahman, M.M., Rahman, H., Murugkar, H. V., Kumar, A., Bujarbaruah, K.M. 2005. Molecular characterization of Escherichia coli O149 and O157serotypes isolated from piglets and poultry. Indian J. Animal Sci. 75(6): 601-605.

Stephen, M.O., Kobayashi, J.M., Lewis, J.H. 1989. Infections with Escherichia coli 0157:H7 in Washington State. J. American. Med. Asso., 262: 355-359.

Tamparo and Carol 2011. Fifth Edition: Diseases of the Human Body. Philadelphia, PA: F.A. Davis Company. p.61.

The Center for Food Security and Public Health 2016. Enterohemorrhagic Escherichia coli and Other E. coli Causing Hemolytic Uremic Syndrome.

Wallet, F., Loiez, C., Renaux, E., Lemaitre, N., Courcol, R. J. 2005. Performance of VITEK2 colorimetric cards for identification of Gram positive and Gram negative bacteria. J. Clin. Microbiol. 43(9):4402-4406.

WHO (World Health Organization) (2016):E. coli O157 Fact Sheets Updated October 2016.

Woodward, M.J., Best, A., Sprigings, K.A., Pearson, G.R., Skuse, A. M., Wales, A., Hayes, C.M., Roe, J.M., Low, C.J., La Ragione, R.M. 2003. Escherichia coli O157:H7 strain NCTC12900 causes attaching–effacing lesions and eae-dependent persistence in weaned sheep. Int. J. Med. Microbiol. 293: 299–308.

Wu, C.J., Hsueh, P.R., Ko, W.C. 2011. A new health threat in Europe: Shiga toxin-producing Escherichia coli O104:H4 infections. J. Microbiol.Immunol. Infect 44 (5): 390–393.

Xiong, Y., Wang, P., Lan, R. 2012. A novel Escherichia coli O157:H7 clone causing a major hemolytic uremic syndrome outbreak in China, PLoS ONE.7 (4):1-10

Yan,Y., Shi, Y., Cao, D., Meng, X., Xia, L., Sun, J.2011. Prevalence of Stx phages in environments of a pig farm and lysogenic infection of the field E. coli O157 isolates with a recombinant converting phage. Current Microbiol. 62(2):458– 464.