Iran. J. Ichthyol. (Special Issue 2023): 172-180

P-ISSN: 2383-1561; E-ISSN: 2383-0964
http://www.ijichthyol.org

Research Article

Isolation and identification of non-O157 Shiga toxin *Escherichia coli* from humans and calves using conventional and molecular technique

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Abstract: This study was done to isolate and yfitnedi the non-O157 STEC in Karbala Province, Iraq. The results showed a new serotype of this bacteria (IQ1) showing different clinical symptoms such as fever, dehydration, colic, fatigue, nausea, abdominal cramps, nerves signs, renal failure, hemolytic uremic syndrome (HUS), hemolytic colitis, bloody to purulent diarrhea in human and animals infected by this pathogen. A total of 400 samples were collected from children's stool and calves' fecal samples, then cultured on different mediums for isolation. The biochemical tests were used for identification such as oxidase, citrate, urease and Voges–Proskauer, catalase, Methyl red, and indole tests. The PCR technique was used for the confirmation of the non-O157 STEC. The results showed from 197 calves' samples 25 (12.69%) and from 203 children's samples, 20 (9.85%) gave positive results. The antibiotic susceptibility test was used to know which antibiotic has the best results and the results revealed Ciprofloxacin with the best zone of inhibition compared with other antibiotics.

Keywords: Non-O157 STEC, PCR, Calves, Human.

Citation: Abdullah, B.F.; Kadhim, K.S. & AL-Shemmari, I.J. 2023. Isolation and identification of non-O157 Shiga toxin *Escherichia coli* from humans and calves using conventional and molecular technique. Iranian Journal of Ichthyology (Special Issue 1): 172-180.

Introduction

The genus *Escherichia* includes *E. coli*, which is a member of Enterobacteriaceae. The gram-negative, facultative anaerobic, non-sporulating bacteria *E. coli* can be found in the environment, intestines of animals, and in extra-intestinal locations (Torres 2004). Shiga toxin-producing *E. coli* (STEC) can be classified into two categories of O157 and non-O157 serogroups based on their O surface antigen type; about 400 non-O157 heterogeneous serogroups had been identified, and about 100 serogroups had been associated with human gastrointestinal infection (Vishram et al., 2021). Six most prevalent non-O157 serogroups recognized by the Centers for Disease Control and Prevention (CDC), which recovered in their surveillance and the detection of both O157 and non-O157 STEC serogroups recommended by clinical laboratories techniques like stool culture methods (Gould et al. 2013). The *stx* genes that are carried by many non-O157 serogroups are the reason for severe human illness (Sears 2006). For many years, the non-O157 STEC has been underestimated clinically due to the lack of specific diagnostic tests (Valilis et al. 2018). However, culture-independent molecular assays were developed recently to detect the virulence genes like *stx*. These assays discovered many infections attributed to non-O157 STEC worldwide (Parsons et al. 2016). HUS and death clinical diseases of non-O157 STEC strains are frequently identified as the major pathogen in both sporadic cases and clinical outbreaks of infection (Bielaszewska et al. 2013, European Centre for Disease Prevention and Control 2020). Hence, this study was done to isolate and identify the non-O157

STEC in Karbala Province, Iraq from the first of December to the end of August 2022.

Materials and methods

After detecting the clinical signs which were nausea, fever, fatigue, dehydration, diarrhea, sometimes bloody diarrhea, abdominal cramps, tiredness, purulent or bloody diarrhea, uncomfortable, and colic, we collected diarrheal samples from calves and children and began cultivating the various culture media. We activated the bacteria on Brain Heart broth, then cultured them on MacConkey Sorbitol agar, which gave bright pink or colorless colonies. After that, we cultured them on EMB agar, which gives a bright metallic green color. We confirmed the type of bacteria using chrome agar medium which gives an inky blue color. The confirmation occurred y using PCR and after that sent for sequencing the serotype of non-O157 STEC bacteria.

Isolation of bacterial genomic DNA was carried out rapidly with the use of the genomic DNA purification ZR Fungal/Bacterial/ Yeast DN MiniPrepTM. After extraction, DNA fragments were analyzed using electrophoresis, and PCR findings were identified using standard DNA to differentiate the bundle size of PCR contact with the agarose gel. Forward 5'-AGA GTT TGA TCC TGG CTC AG-3' 1250, Reverse 5'-GGT TAC CTT GTT ACG ACTT-3', was the primer employed (Lane 1991). Canada's 16s ribosomal RNA gene was analyzed which is a 1250 bp section of the gene (Canadian Integrated DNA Technologies). The lyophilized primers were reconstituted in free DdH_2O to a concentration of 100pmol/ l as stocks solution, and the stocks were stored at -20°C until ready to be suspended in the reaction mixture (Lane 1991).

Denaturation at 94°C for 45 seconds, annealing at 56°C for 45 seconds, extension at 72°C for 1 minute, and final extension at 72°C for 7 minutes made up the 35-cycle sequence. A thermal cycler was used for the amplification (PCR). It all started with the making of a red agarose gel. A gel documentation system was used to record the results of the electrophoretic detection of the DNA band. All the components of a polymerase chain reaction.

To determine the MIC, we used the procedure described by Vandepitte et al. (2003). A bacterial culture is started by injecting the bacteria into tryptone soya broth and incubating it at 37°C for 24 hours. The bacteria are streaked onto a plate of tryptone soy agar and given another 24 hours to proliferate. By comparing the turbidity of the tube to the 0.5 McFarland standard and adjusting the density of the test suspension to that of the standard, by adding more bacteria or more sterile saline, we were able to calculate the bacterial concentration of $1.5x10^8$CFU/ml.

Muller Hinton agar plates were inoculated by dipping sterile swabs into inoculum, removing excess inoculum by pressing and rotating the swab against the side of the tube above the level of the liquid, then the swab streaked all over the surface of the medium. After letting the inoculum dry for a few minutes at room temperature with the lid closed, wells were punched into the Muller Hinton agar plates (penicillin, azithromycin, oxacillen, lincomycin, oxytetracycline, siprofloxacin, gentamicin, and condalab). The plates were incubated at 37°C for 24 hours. The findings were calculated using a ruler and millimeter units to measure the diameters of the inhibition zones surrounding the wells displaying an inhibitory effect (Moreira et al. 2007).

Results

MacConkey Sorbitol agar: *Escherichia coli* sample on Maconky sorbitol ager appeared bright pink or colorless colonies after 24 hours of incubation (Fig. 1).

Eosine Methylene blue agar: Bacterial colonies that appear bright pink or colorless colonies on Maconky sorbitol media were picked up by loop and cultivated on the EMB resulting in a green color with a distinctive metallic sheen that appears on the surface of the bacteria colonies when exposed to light (Fig. 2).

Chrom agar *E. coli* O157: After using the EMB

Fig.1. Non O-157 STEC on Macconkey Sorbitol Agar.

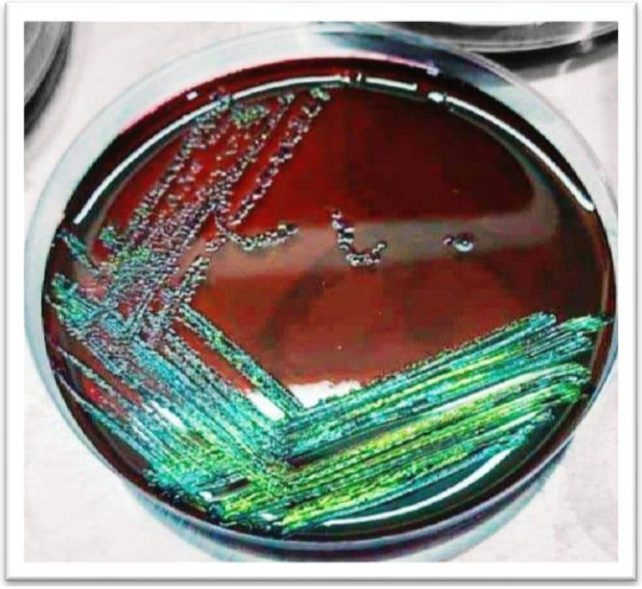

Fig.2. The colonies of non O-157 STEC cultivated on EMB Agar.

media, we applied the chrome ager as a selective media to differentiate between non-O157 STEC and other types of *E coli* which appear as blush green in color colonies after incubated for 24 hours (Fig. 3).

Biochemical test of Non-O-157 STEC: Biochemical tests used to conformation the infection, gave negative results for the oxidase test, citrate, ureas, and Vogas–Proskauer, while positive results appeared in catalase, methyl red test, and indole test.

DNA extraction result: The 16s RNA gene was used for the amplification, yielding a product size of around 1250 bp that was deposited in the Genebank under the accession number LC738862.

Antibiotic susceptibility test: According to the standard procedures, antimicrobial susceptibility tests were done on Mueller-Hinton agar (Oxoid, Hampshire, England) using the Kirby Bauer disk diffusion method. The antimicrobial agents tested were: tetracycline (30µg), erythromycin (15µg), gentamicin (10µg), ciprofloxacin (5µg), amoxicillin (10µg), penicillin, azithromycin, oxacillin, and candalab and the best antibiotic with a wider zone of

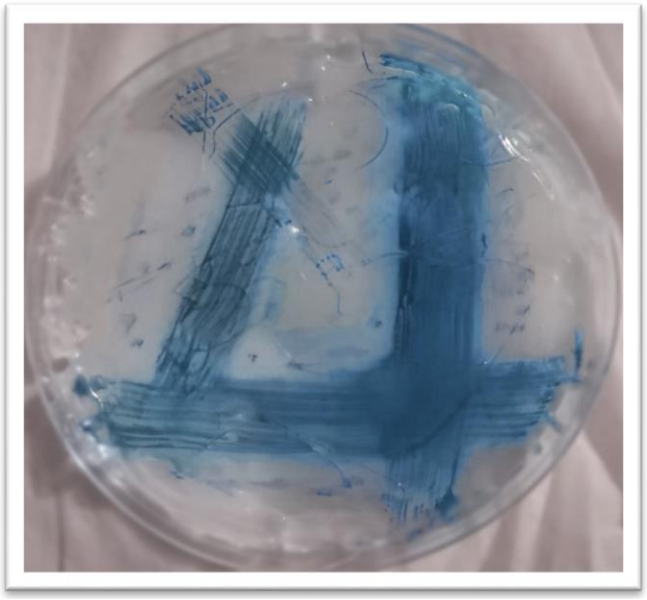

Fig.3. The colonies of Non O-157 STEC on Chrom agar.

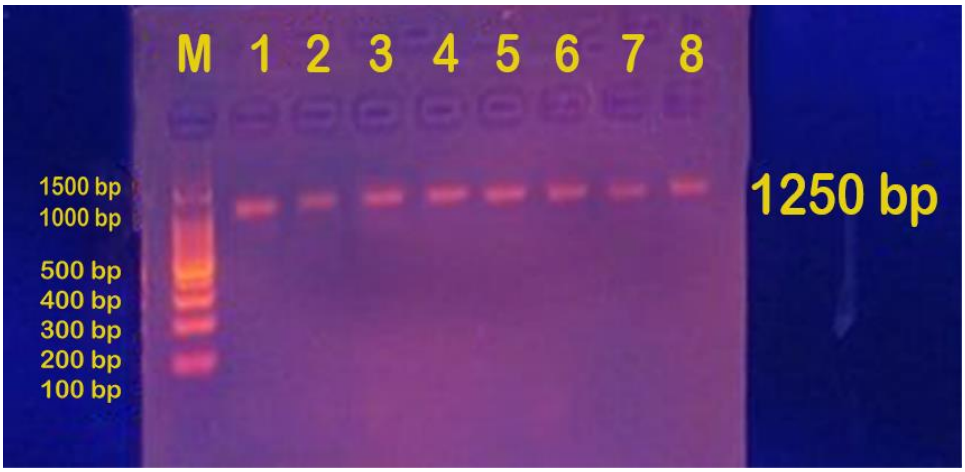

Fig.4. PCR result showing the product of Non-157 STEC of isolation 1-8 sample of children and calve fecal sample using 16s RNA genes with size of 1250 bp.

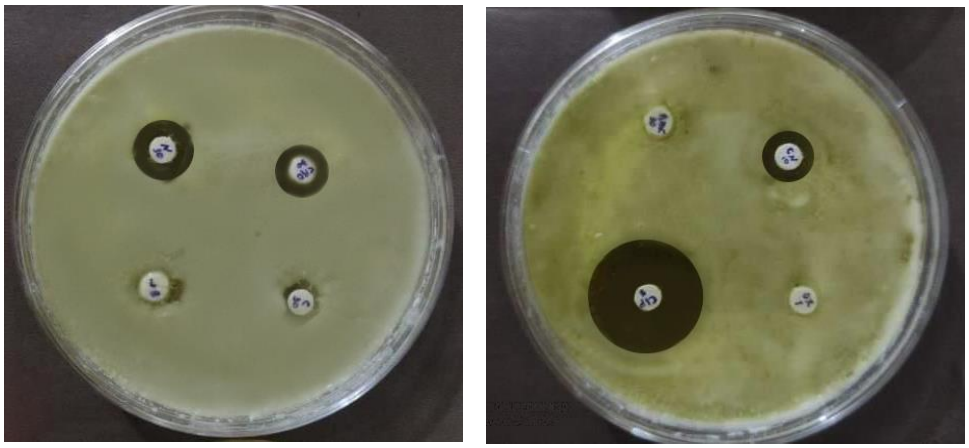

Fig.5. The sensitivity test result using different antibiotics (tetracycline, erythromycin, gentamicin, ciprofloxacin, amoxicillin, azithromycin, and oxacillin).

inhibition was found in ciprofloxacin.

Discussion

The non-O157 STEC are important zoonotic pathogens, with the potential to cause severe gastrointestinal disease that can lead to a variety of sequelae, including HC, HUS, and TTP (Karmali et al. 2004). The purpose of this study was to determine the prevalence of non-O157 STEC in children and calves in Karbala Province, Iraq. Serogroup (IQ1) was the only isolated serotype in children and calves in our study. The nonO157 STEC is one of the most common pathogens that cause infections in humans.

Clinical signs: The clinical symptoms caused by the food-borne bacterium non-O157 STEC included mild to severe watery to bloody diarrhea and hemolytic colitis HC and other symptoms as mentioned in the results and this agrees with that appear reported cases (Dean et al. 2013). Other studies showed signs of the hemolytic uremic syndrome (HUS) and renal failure (Todar et al. 2007) and the bacterium acts systemically on sensitive cells in the kidneys, intestine, and other organs (William et al. 2007). Disseminated intravascular coagulation (DIC) and thrombotic thrombocytopenic purpura (TTP) are possible complications in the elderly and young (Gyles et al. 2007). EHEC O157:H7 strains of *E. coli* are particularly virulent and account for the vast majority of bacterially-caused HUS cases across the world (George et al. 2005). Healthy domesticated ruminants, most notably cattle (William et al. 2007), but also sheep and probably goats (Serna & Boedeker 2008), are the primary animal carriers.

Rate of infection: According to our findings, the detected serotype in this study of non-O157 STEC isolates showed a rate of infection of about 20% of isolates. Many studies detected other serogroups like O26, O111, and O103 made about 50% of the non-O157 STEC found in the samples and similarly with that found in the STEC O157 infections that were commonly isolated from children (Parry et al. 2000; Slutsker et al. 1997). The non-O157 STEC showed a summer seasonality and was more commonly isolated from children. In addition, other works have shown that STEC is more often from cow feces in the summer than in the winter and that human sickness is also more common in the summer (Nataro et al. 1998; Gyles et al. 2006; Sami Jarad 2016).

Culture medium

Culturing on Sorbitol MacConky Aga: Colonies of bacteria collected using a loop and cultured on a SMAC for 24 hours before being seen, showed that non-O157 STEC colonies were colorless while other *E. coli* colonies were bright pink. Other studies on children's stool isolates grew on SMAC medium and looked colorless as sorbitol fermenting isolates. Differentiating EHEC from another *E. coli* serotype was accomplished using the medium. For the majority of EHEC serotypes, SMAC had a probability of 23.5%, while the likelihood of identifying O157:H7 alone was 50% (Novicki et al. 2000). The majority of EHEC serotypes cannot ferment D-sorbitol, and the resultant colonies on the SMAC are colorless. The growth of gram-positive bacteria can be slowed using bile salts or crystal violet (Becton Dickinson 2013).

Culturing on EMB: To identify *E. coli* from the wide variety of other enteric bacteria species that can be present in human and animal feces, either as pathogenic organisms or as normal flora, the isolates were cultured and incubated on an EMB medium. Due to the rapid lactose fermenters *E. coli* producing acid, the pH was reduced, allowing dye absorption by colonies, resulting in a green color with a distinctive metallic sheen when exposed to light that appears on the surface of the bacteria colonies grown in EMB medium (Xavier et al. 2016). This is because the EMB medium contains the dyes eosin and methylene blue, which have metachromatic characteristics. The EMB agar dyes, eosin Y and methylene blue serve as pH indicators and inhibitors of gram-positive bacteria, respectively (Leininger et al. 2001). The poisonous dyes used in EMB limit the growth of Gram-positive bacteria and certain yeast while allowing Gram-negative bacteria to flourish. Hence, the presence of enteric bacteria in this medium will

differ according to whether or not they are capable of fermenting lactose in addition to sucrose; a metallic sheen will develop on *E. coli* (Wanger et al. 2017).

Culturing on CHROM O157agar: Individual colonies with green metallic sheen color on EMB agar were hand-picked and re-cultured on CHROM O157agar. Blue-green colonies indicated that the STEC strains were not O157. The samples from children and from calves grew on Chrom agar medium were blue-green color colonies after incubation. This medium is so important because it can differentiate the diagnosis of non-O157 STEC from other *E. coli* either by changing color or by inhibiting other pathogens. For that chromogenic agar is considered a perfect choice for the detection of bacteria (Chui et al. 2018). The screening and detection of non-O157 STEC isolate using this medium are enhanced by its fluorescence property, allowing for the detection of STEC co-infections (Hirvonen et al. 2012). Substrates uniquely identified by -D-galactosidase and -D-glucuronidase to differentiate non-O157 STEC from other *E. coli* strains (Hussein & Bollinger 2008).

Biochemical tests for identification *E. coli*: The biochemical tests were performed for more conformation to the isolates, lack of Cytochrome oxidase enzyme caused the absence of color change in an oxidase test, while the presence of the catalase enzyme gave a positive result in a catalase test which acts to the breakdown of hydrogen peroxide into oxygen and water (Tenaillon et al. 2010). The presence of a rose ring in the upper test tube in the methyl red test made a positive result by generating a mixed acid fermenter and in the indole test, the production of indole from tryptophan by tryptophanase. The negative results given by the urease and simmon citrate tests due to the *E. coli* does not depend on citrate as its only carbon source (Mac 1980). In order to distinguish between intestinal bacteria, IMIVIC tests were utilized (Family Enterobacteriaceae). Among these are the indole test, the methyl red test, and the Voges Proskauer and citrate test; the latter yielded a negative result in agreement with Ramadan's (2017) findings, as citrate is not a suitable carbon source for *E. coli*. The indole test showed that *E. coli* could convert tryptophan into indole, and the resulting rose ring was in the upper test tube. Lack of color development during the oxidase test revealed that the *E. coli* sample tested did not contain Cytochrome oxidase, whereas a positive result in the catalase test suggested that the bacteria were able to convert hydrogen peroxide into water and oxygen. The lack of urease enzyme caused a negative urease test, which indicates that the urea was not hydrolyzed (Quinn et al. 2004).

Genomic DNA extraction of non-O157 STEC: Genomic DNA from bacteria was quickly isolated using the ZR Fungal/Bacterial/Yeast DN MiniPrep, a genomic DNA purification kit. Using the 16s RNA gene, we were able to successfully amplify a product of around 1250 base pairs. We were able to validate the presence of *E. coli* genes in eight different stool samples using the PCR technique. The sequencing of 8 canine stool isolates and comparison to GenBank for homology (Ahmed et al. 2021).

Antibiotic Susceptibility Test: Overall, *E. coli* in this investigation showed a significant level of resistance to the antibiotics used in this study. These results agree with those of the previous studies (Orrett et al. 2001). This study found greater resistance rates than Khan et al. (2002), but Iqbal & Patel (2002) and Okonko et al. (2009) found lower resistance Andargachew et al. (2006) found that *E. coli* develops a high degree of resistance to tetracycline, while Petkovs¡ek et al. (2009) found that *E. coli* has a high level of resistance to erythromycin. Resistance rates for *E. coli* to erythromycin, amoxicillin, and tetracycline were all above 80% in clinical samples in our work that are consistent with those of Briscoe et al. (2005) and Bharathi et al. (2008). In contrast to susceptibility patterns described in earlier studies (Barrett et al. 1999; Karlowsky et al. 2002; Zhanel et al. 2006), the antimicrobial resistance rates observed in the current investigation were much greater.

Gentamicin, ciprofloxacin, and chloramphenicol were all effective against the *E. coli* isolates tested. Comparable susceptibility rates have been found in investigations performed in both Ethiopia (Tesfaye et al. 2009) and Nigeria (Wariso et al. 2006). Studies in Nigeria and India (Bharathi et al. 2002; Wariso et al. 2006) have found that bacteria there are very sensitive to the antibiotic's ciprofloxacin, gentamicin, and norfloxacin. The most effective antimicrobials in this investigation against *E. coli* isolates were ciprofloxacin.

Conclusions

The non-O157 STEC is an important zoonotic disease that causes bloody to purulent diarrhea and other clinical signs in calves and humans. The new serotype of non-O157 STEC (IQ1) which was isolated from calves and humans in our study has severe pathogenic effects. The chrome ager and PCR were helpful for the isolation and identification of the non-O157 STEC. The susceptibility test revealed that ciprofloxacin gave a wider zone of inhibition.

References

Barrett, S.P.; Savage, M.A.; Rebec, M.P.; Guyot, A.; Andrews, N. & Shrimpton, S.B. 1999. Antibiotic sensitivity of bacteria associated with community-acquired urinary tract infection in Britain. Journal of Antimicrobial Chemotherapy 44(3): 359-365.

Becton Dickinson, 2013. Bula BD MacConkey Agar with Sorbitol. 4.

Bharathi, M.J.; Ramakrishnan, R.; Maneksha, V.; Shivakumar, C.; Nithya, V. & Mittal, S. 2008. Comparative bacteriology of acute and chronic dacryocystitis. Eye 22(7): 953-960.

Bharathi, M.J.; Ramakrishnan, R.; Vasu, S.; Meenakshi, R. & Palaniappan, R. 2002. In-vitro efficacy of antibacterials against bacterial isolates from corneal ulcers. Indian Journal of Ophthalmology 50(2): 109-114.

Bielaszewska, M.; Mellmann, A.; Bletz, S.; Zhang, W.; Köck, R.; Kossow, A.; Prager, R.; Fruth, A.; Orth-Höller, D.; Marejková, M.; Morabito, S.; Caprioli, A.; Piérard, D.; Smith, G.; Jenkins, C.; Čurová, K. & Karch, H. 2013. Enterohemorrhagic *Escherichia coli* O26:H11/H−: a new virulent clone emerges in Europe. Clinical Infectious Diseases 56(10): 1373-1381.

Briscoe, D.; Rubowitz, A. & Assia, E.I. 2005. Changing bacterial isolates and antibiotic sensitivities of purulent dacryocystitis. Orbit 24(1): 29-32.

Chui, L.; Christianson, S.; Alexander, D.C.; Arseneau, V.; Bekal, S.; Berenger, B. & Ziebell, K. 2018. CPHLN recommendations for the laboratory detection of Shiga toxinproducing Escherichia coli (O157 and non-O157). Canada Communicable Disease Report= Releve des Maladies Transmissibles au Canada 44(11): 304-307.

Dean, Z. 2013. Shiga toxin producing Escherichia coli (STEC) in cattle: factors affecting fecal shedding of *E. coli* O157:H7 and detection methods of non-O157 STEC. Dissertation, Department of Diagnostic Medicine/Pathobiology. Kansas State University.

European Centre for Disease Prevention and Control. 2020. Shiga-toxin/verocytotoxin-producing Escherichia coli (STEC/VTEC) infection. ECDC annual epidemiological report for 2018. ECDC, Stockholm, Sweden.

George, J. 2005. Thrombotic Thrombocytopenic purpura, and Haemolytic Uremic Syndrome. Current Hematological Reports 4:167-169.

Getachew, T.; Asrat, D.; Yimtubezinash, W. & Messele, G. 2009. Microbiology of discharging ears in Ethiopia. Asian Pacific Journal of Tropical Medicine 2(1): 60-67.

Gould, L.H.; Mody, R.K.; Ong, K.L.; Clogher, P.; Cronquist, A.B.; Garman, K.N. & Griffin, for the Emerging Infections Program FoodNet Working Group, P.M. 2013. Increased recognition of non-O157 Shiga toxin–producing Escherichia coli infections in the United States during 2000–2010: epidemiologic features and comparison with *E. coli* O157 infections. Foodborne Pathogens and Disease 10(5): 453-460.

Gyles, C.L. 2006. Shiga toxin-producing *Escherichia coli*: a review. Journal of Animal Science 85: 45-62.

Gyles, C.L. 2007. Shiga toxin-producing Escherichia coli: an overview. Journal of Animal Science 85: 45-62.

Hirvonen, J.J.; Siitonen, A. & Kaukoranta, S.S. 2012. Usability and performance of CHROMagar STEC medium in detection of Shiga toxin-producing

Escherichia coli strains. Journal of clinical microbiology, 50(11), 3586-3590

Hussein, H.S. & Bollinger, L.M. 2008. Influence of selective media on successful detection of Shiga toxin–producing *Escherichia coli* in food, fecal, and environmental samples. Foodborne Pathogens and Disease 5(3): 227-244.

Iqbal, M.K. & Patel, I.K. 2002. Susceptibility patterns of *Escherichia coli*: Prevalence of multidrug-resistant isolates and extended spectrum beta-Lactamase phenotype. The Journal of the Pakistan Medical Association 52: 407-417.

Johnson, K.E.; Thorpe, C.M. & Sears, CL. 2006. The emerging clinical importance of non-O157 Shiga toxin-producing *Escherichia coli*. Clinical Infectious Diseases 43: 1587-1595.

Karlowsky, J.A.; Kelly, L.J.; Thornsberry, C.; Jones, M.E. & Sahm, D.F. 2002. Trends in antimicrobial resistance among urinary tract infection isolates of *Escherichia coli* from female outpatients in the United States. Antimicrobial Agents and Chemotherapy 46(8): 2540-2545.

Karmali, M.A.; Petric, M.; Lim, C., Fleming, P.C.; Arbus, G.S. & Lior, H. 2004. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. The Journal of Infectious Diseases 189(3): 556-63

Khan, N.A.; Saba, N.; Abdus, S. & Ali, A.Q. 2002. Incidence and antibiogram patterns of *E. coli* isolates from various clinical samples from patients at NIH Islamabad. Pakistan Journal of Biological Sciences 1: 111-113.

Leininger, D.J.; Roberson, J.R. & Elvinger, F. 2001. Use of eosin methylene blue agar to differentiate Escherichia coli from other gram-negative mastitis pathogens. Journal of Veterinary Diagnostic Investigation 13(3): 273-275.

Mac, F.J.F. 1980. Biochemical Tests for Identification of Medical Bacteria. pp: 173-183. Baltimore: Williams and Wilkins Co.

Mulu, A.; Moges, F.; Tessema, B. & Kassu, A. 2006. Pattern and multiple drug resistance of bacterial pathogens isolated from wound infection at University of Gondar Teaching Hospital, Northwest Ethiopia. Ethiopian Medical Journal 44(2): 125-131.

Nataro, J.P. & Kaper, J.B. 1998. Diarrheagenic *E. coli*. Clinical Microbiology Reviews 11(1): 142-201.

Novicki, T.J.; Daly, J.A.; Mottice, S.L. & Carroll, K.C. 2000. Comparison of sorbitol MacConkey agar and a two-step method which utilizes enzyme-linked immunosorbent assay toxin testing and a chromogenic agar to detect and isolate enterohemorrhagic Escherichia coli. Journal of Clinical Microbiology 38(2): 547-551.

Okonko, I.O.; Soleye, F.A.; Amusan, T.A.; Ogun, A.A.; Ogunnusi, T.A.; Ejembi, J. & Onajobi, B.I. 2009. Incidence of multi-drug resistance (MDR) organisms in Abeokuta, Southwestern Nigeria. Global Journal of Pharmacology 3(2): 69-80.

Orrett, F.A. & Shurlandl, S.M. 2001. Prevalence of Resistance to Antimicrobials of *Escherichia coli* Isolates fTrom Clinical Sources at a Private Hospital in Trinidad. Japanese Journal of Infectious Diseases 54: 64-68.

Parry, S.M. & Palmer, S.R. 2000. The public health significance of VTEC O157. Symposium series (Society for Applied Microbiology) 88(Suppl): S1-S9.

Parsons, B.D.; Zelyas, N.; Berenger, B.M. & Chui, L. 2016. Detection, characterization, and typing of Shiga toxin-producing Escherichia coli. Frontiers in Microbiology 7: 478.

Petkovšek, Z.; Eleršič, K.; Gubina, M.; Zgur-Bertok, D. & Starčič Erjavec, M. 2009. Virulence potential of *Escherichia coli* isolates from skin and soft tissue infections. Journal of Clinical Microbiology 47(6): 1811-1817.

Quinn, P.J.; Carter, M.E.; Markey, B. & Carter, G.R. 2004. Clinical Veterinary Microbiology. Mosby Edinburgh, London, New York, Oxord and Philadephia. USA. pp: 21- 63

Rawaa Sadi, A. 2021. Comparative Treatment Study among Colicin E1 EnterocinA1 and Enrofloxacin of Non-O157 Shiga Toxin-Producing *E. coli* Experimentally in Rabbits That Isolated from Children and Dogs. University of Baghdad.

Sami Jarad, A. 2016. Bacteriological, Immunological and Pathological Study on Mice Experimentally Infected with NonO157 Shiga Toxin Producing *E.coli* Isolated from Children and Calves. Baghdad University

Serna IV, A. & Boedeker, E.C. 2008. Pathogenesis and treatment of Shiga toxin-producing Escherichia coli infections. Current opinion in gastroenterology 24(1):

38-47.

Slutsker, L.; Ries, A.A.; Greene, K.D.; Wells, J.G.; Hutwagner, L. & Griffin, P.M. 1997. Escherichia coli O157: H7 diarrhea in the United States: clinical and epidemiologic features. Annals of Internal Medicine 126(7): 505-513.

Tenaillon, O.; Skurnik, D.; Picard, B. & Denamur, E. 2010. The population genetics of commensal *Escherichia coli*. Nature Reviews Microbiology 8(3): 207-217.

Todar, K. 2007. Pathogenic *E.coli*. online textbook of bacteriology. University of Wisconsin-Madison, Department of Bacteriology, pp: 11- 30.

Torres, A.G.; Knack, K.J.; Tutt, C.B.; Popov, V. & Kaper, J.B. 2004. Characterization of the second long polar (LP) fimbriae of *Escherichia coli* O157:H7 and distribution of LP fimbriae in other pathogenic E. coli strains. FEMS Microbiology Letters 238(2): 333-344.

Valilis, E.; Ramsey, A.; Sidiq, S. & DuPont, H.L. 2018. Non-O157 Shiga toxin-producing *Escherichia coli*-a poorly appreciated enteric pathogen: systematic review. International Journal of Infectious Diseases 76: 82-87.

Vishram, B.; Jenkins, C.; Greig, D.R.; Godbole, G.; Carroll, K.; Balasegaram, S. & Byrne, L. 2021. The emerging importance of Shiga toxin-producing *Escherichia coli* other than serogroup O157 in England. Journal of Medical Microbiology 70(7).

Wanger, A.; Chavez, V.; Huang, R.; Wahed, A.; Dasgupta, A. & Actor, J. K. 2017. Microbiology and molecular diagnosis in pathology: a comprehensive review for board preparation, certification and clinical practice. Elsevier Science Publishing Co Inc. USA

Wariso, B.A. & Ibe, S.N. 2006. Bacteriology of chronic discharging ears in Port Harcourt, Nigeria. West African Journal of Medicine 25(3): 219-222.

William, S. 2007. Haemolytic Uremic Syndrome H.U.S., American Academy of Emergency Medicine 54: 664-669.

Xavier, P.D.; Vijay, S.T.; Ramya, V.; Frankantony, P.B.; Priyanka, K.S. & Shiny, J. 2016. Metachromasia and Metachromatic Dyes: A review. International Journal of Advanced Health Sciences 2: 14-20.

Zhanel, G.G.; Hisanaga, T.L.; Laing, N.M.; DeCorby, M.R.; Nichol, K.A.; Weshnoweski, B. & NAUTICA, Group. 2006. Antibiotic resistance in *Escherichia coli* outpatient urinary isolates: final results from the North American Urinary Tract Infection Collaborative Alliance (NAUTICA). International Journal of Antimicrobial Agents 27(6): 468-475.