Using PCR technique for diagnosis of bacterium *Escherichia coli* O157:H7 isolated from urine samples of humans and sheep

Al-wgaa, A. A.[@] and Alwan, M. J.
Zoonosis unit, College of Veterinary Medicine, Baghdad University, Iraq.
E-mail: dr_alaa_alwgaa@yahoo.com
Accepted on: 2/3/2014

Summary

The aims of the current study were to determine the percentages of *E.coli* O157:H7 in the urinary tract infections (UTIs) of humans and in the urine of apparently healthy sheep, and to determine the genotype of the isolates by PCR assay. Two hundred and twenty eight urine samples were collected from young children and adult patients of both sexes suffering from UTIs during a period December 2012 to the end of March 2013. And randomly collected 75 urine samples from apparent healthy sheep of both sexes that were slaughtered in AL Shoela, AL Rahmanea Slaughterhouse and College of Veterinary Medicine field in Baghdad the end of February to half of April 2013. All urine samples were incubated aerobically at 37°C for 24-48 hrs on blood agar, MacConkey agar as well as special media and the isolates were identified by biochemical tests, then the isolates were confirmed and diagnosed by PCR assay. The results showed that of human urine samples ,8 samples were *E. coli* O157:H7 positive isolates(3.50%) ,young children expressed (4) isolates of *E.coli* O157:H7 as compared with those in adult (4 isolates) and the percentage of these isolates in females were (2.6%) as compared with those in males (0.87%). Also the current study demonstrated that all isolates culturing positive serotype of *E.coliO* 157:H7 were positive by PCR assay .The genes of eaeA ,hly and Stx2 were recorded in 7,8 and 2 serotype of *E.coliO157*:H7 respectively. The result revealed that 21(28%) out 75 sheep urine samples were *E.coli* positive isolates, 13 out of 75 samples were *E.coli* O157:H7 positive isolates. The percentage of isolates from ram urine samples was (14.66%) as compared to those samples of ewes (2.66%).The result also recorded that 10,13,12,4 and 3 of bacterial isolates of sheep urine samples carried eaeA, hlyA, hlyA plasmid,Stx1 and Stx2 genes respectively. In conclusion the healthy sheep in Baghdad city, harbor shiga -toxin producing *E.coliO157:H7* in their urinary tracts and these organism induced UTIs associated with renal failure in the humans and carried the same virulent genes that reported in the sheep isolates.

Keywords: ***Escherichia coli* O157:H7, PCR, Laboratory technique.**

Introduction

Generally *Escherichia coli* is considered a part of non-pathogenic intestinal normal flora of humans and animals. In certain strain of this organism can cause intestine and extraintestinal infections via its ability to get genes that enable them to become pathogen. According to the virulence factors and mechanisms by which the pathogenic strain *E.coli*, these microorganisms are divided into pathotypes, and one of these pathotype is Shiga toxin (STEC) or *Escherichia coli* O157:H7, which is considered a public health problem bacteria which induced food-borne diarrhoes, bloody diarrhea and hemolytic uremic syndrome that occurring with average in 4% of infection cases in young children in addition to infecting adult individuals (1). Also these organisms are responsible for different outbreaks in animals, poultry and humans worldwide (2). Urinary tract infections (UTI) caused by *E.coli*O157:H7 is one of the most important diseases in infants and children that may be the cause of renal failure or may lead to death of the patients (3).

Cattle and sheep are considered the primary reservoir for *E. coli* O157:H7 and these animals carry *E.coli* O157 without clinical symptoms and can passively shed these organisms in their feces for long periods (4), therefore they are considered the important sources of food-borne and direct animal contact infections (5). There are no protective vaccine against *E.coli* O157:H7, therefore, it must be rapidly diagnoses in order to take treatment and prevent renal damage. The rapid

specific and sensitive confirming diagnostic method of these organs is PCR assay (6).

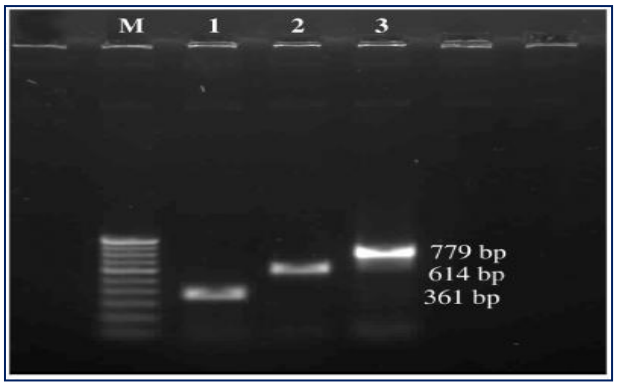
In Iraq, there is limited information on the urinary tract infection by *E.coli* O157 in humans and sheep as well as genotypic feature of these organism ,therefore the aim of the current study is to determine the percentage of urinary tract infection of humans and sheep with *E.coli* O157:H7 by using both PCR assay and culturing method.

Materials and Methods

In this study, general and selective media were used to isolated *E.coli*O157:H7 with pure culture according to (7 and 8). Two hundred and twenty eight humans urine samples were collected from young and adult patients of both sexes suffering from UTIs during the period December 2012 to the end of March 2013. In addition to randomly collected 75 urine sample from apparent healthy sheep both sexes that were slaughtered in AL Shoela, AL Rahmanea slaughterhouse and College of Veterinary Medicine field in Baghdad the end of February to half of April 2013. All urine samples were incubated aerobically at 37°C for 24- 48hrs on blood agar, MacConkey agar according to (7) as well as special media(Sorbitol MacConky agar, Chrom agar *E.coli* O157) according to (8) and the isolates were identified by biochemical tests like IMViC (Indole , Methyl Red, Voges Proskeur and Citrate utilization) test according to (7), then the isolates were confirmed in diagnosis by PCR assay using four primers to amplify enterohaemolysin (hlyA) gene (9), Shiga toxin 1(Stx1) gene , Shiga toxin 2(Stx2) gene (10) and intimin (eaeA) gene (11). DNA extraction was done by using (Presto™ Mini g DNA Bacteria Kit Geneaid. USA), PCR master mix reaction was prepared by using AccuPower PCR premix Kit. In addition analyses the PCR product by agarose gel electrophoresis (Fig.1).

Results and Discussion

Bacterial colonies appeared as rosy pink color during 24-48 hr post-incubation on McConky media at 37°C and a metallic green sheen color of colonies on Eosin methylene blue agar. Also the bacterial isolates showed gram-negative motile bacilli according to



Figure, 1: Results of the PCR assay
Lane M: 100-bp DNA marker
Lane 1: amplifying 361-bp segment of hly A
Lane 2: amplifying 614-bp segment of stx 1
Lane 3: amplifying 779-bp segment of stx 2

microscopically appearance, morphology of the colonies, biochemical tests and numerical profile in API-20E system. It was indicated that the bacterial isolates were *E.coli*. Smooth colorless colonies appearing on selective media Cefixime Tellurite-Sorbitol MacConkey agar and mauve color on Chrom agarO157. It was indicated that the bacterial isolates were *E.coli* O157, and these strain were confirmed in diagnosis as *E.coli*O157:H7 serotype by PCR assay.

The present result showed that 8 out 228 (3.50%) human urine samples were *E.coli* O157:H7 positive isolates. Also the result revealed that the number of serotype in females showed high percentage of serotype O157:H7 isolates (2.6%) as comparing with those reported in the males (0.87%).The current finding demonstrated that all isolates culturing positive serotype *E.coli* O157:H7 were positive by PCR assay and 7 of them expressed eaeA gene and 8 of these serotype showed hlyA gene and hlyA plasmid gene while only 2 serotypes showed Stx2 gene (Table, 1). The present finding showed that (3.50%) human urine samples expressing serotype O157:H7, these ratios may indicate that *E.coli*O157:H7 play crucial role in urinary tract infection in the humans and these result was gree with result of (12) who detected (2.38%) of *E.coli* isolated from 12572 children urine samples was +ev *E.coli* O157:H7 serotype. Also it was recorded in the present study that the result of PCR assay agreed with the result of bacterial culturing ,these observation, are support the idea that the PCR

assay is a rapid and more sensitive test for diagnosis of these microorganism in contaminated materials. These idea, were in consistent with (13) who explained that the PCR assay was considered to be the most sensitive method that using to know whether a sample contains *E.coli* O157:H7 through detecting the genetic feature of these organism.

The current result of PCR assay detected presence of eae A, hlyA , hlyA plasmid and Stx2 genes in the local isolates *E.coli* O157:H7.These observations may indicated that these serotype may cause renal failure in the infected individuals. These result were in agreement with (14) who showed that *Ecoli* isolated from urinary tract infection expressed gene of shiga toxin, (17.3%) Stx1 (52.45%) Stx2 and (30.3%) both Stx1 and Stx2. Also (15) reported eae A, Stx1 and Stx2 genes in serotype isolated from the human samples by PCR assay. Also (14) recorded 5 out 9 EHEC strains (55%) produced Stx genes that were detected by PCR methods.

Table, 1: Showed results of PCR assay to confirm diagnosis of E.coli O157:H7

No. of isolates	+ev culturing	+ev PCR	eaeA gene	hlyA gene	hlyA plasmid gene	Stx1 gene	Stx2 gene
8	8	8	7	8	8	-	2

The morphological appearance of bacterial colonies as well as microscopic finding of bacterial isolates from sheep urine samples were similar to those strains isolated from human urine samples.(Table, 2) showed that 13 out 75(17.33%) sheep urine samples were positive *E.coli* O157:H7 positive isolates and 11(18.03%) out 61 ram urine samples were +ev serotype O157:H7 and 2 (14.28%) out 14 ewe urine samples were +ev *E.coli* O157:H7.Also the current result showed that the 10 strains of *E.coli* O157:H7 isolates expressed eaeA genes , 13 and 12 of this strains were expressed hlyA and hlyA plasmid respectively ,in addition 4 and 3 of the isolates strain showed Stx1 and Sxt2 respectively (Table, 3). This result may indicate that the strains isolated from urine samples of sheep are shiga toxin producing strains. In addition, these virulence genes detected in this strain were similar to those reported in the same strain isolated from human urine samples. These observations may be indicated that the healthy sheep may serve as important source of human infection by these organisms (16). Also (17) isolated shiga toxin producing E.coli from goats, deer and horses. Moreover, a genotypic study revealed eaeA gene carried by 10 isolates strains. These investigations may indicate that these strains were a potential humane pathogen, the outer membrane protein (intimin) encoded by eaeA genes are essential in the A\E ability of *E.coli* O157:H7 together with shiga toxin that induce host tissue damage, however, the present study is considered the first study in Iraq to determine the genotypic features of *E.coli* O157:H7 strain isolated from urine of the sheep. It was demonstrated that the number of bacterial strains carried eaeA gene (10 isolates) was lower than those strain that carried hlyA and hlyA plasmid. These results agreed with (18) who reported that eaeA gene was less frequently in Ovine STEC strains. Moreover, the study revealed that some strains did not carry eaeA and shiga toxin genes. These observations agreed with (19) who found that some strains of E.coli O157:H7 did not carry Stx or eaeA genes.

Table, 2: Showed number of urine samples from male and female sheep with percentage of *E.coli* O157:H7 isolates.

sex	No. of samples	+ev *E.coli*	+ev serotypeO157:H7	% of O157:H7 isolates
Males	61	5	11	18
females	14	3	2	14.28
Total	75	8	13	17.33

Table, 3: Showed results of PCR assay to confirm diagnosis of *E.coli* O157:H7

No. of isolates	+ev Culturing	+ev PCR	eaeA	hlyA	hlyA plasmid	Stx1	Stx2
13	13	13	10	13	12	4	3

References

1. Kaper, J. B.; Nataro, J. P. and Mobley, H. L. T. (2004). Pathogenic *Escherichia coli*. Nat. Rev. Microbiol., 2:123–140.
2. Deisingh, A. K. and Thompson, M. (2004). Strategies for the detection of *Escherichia coli* O157:H7 in foods. J. Appl. Microbiol., 96:419–429.
3. Mead, P. S.; Slutsker, L.; Dietz, V.; McCaig, L. F.; Bresee, J. S.; Shapiro, C.;Griffin, P. M. and Tauxe, R.V. (1999). Food-related illness and death in the United States. Emerg. Infect. Dis., 5:607-625.
4. Grauke, L. J.; Kudva, I. T.; Yoon, J. W.; Hunt, C. W.; Williams, C. J. and Hovde, C. J. (2002). Gastrointestinal tract location of *Escherichia coli* O157:H7 in ruminants. 68:2269-2270.
5. Hancock, D. D.; Besser, T. E.; Kinsel, M. L.; Tarr, P. I.; Rice, D. H. and Paros, M. G. (1994). The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington State. J. Epidemiol. Infect., 113:199–207.
6. Liu, Y.; Wang, C.; Fung, C. and Li, X. F. (2010). Quantification of viable but nonculturable *Escherichia coli* O157:H7 by targeting the rpoS mRNA. Anal. Chem., 82:2612–2615.
7. Quinn, P. J.; Carter, M. E.; Markey, B. and Carter,G.R. (2004). Clinical Veterinary Microbiology. 6th ed. Mosby an imp. Wolf, London, PP: 66 – 85.
8. Mc Donough, P. L. (2000). Prevalence of *Escherichia coli* O157:H7 from cull dairy cows in New York state and comparison of culture methods used during preharvest food safety investigations. J. Clin. Microbiol., 38: 318-322.
9. Wang, R. F.; Cao, W. W. and cerniglia, C. E. (1997). A universal protocol for PCR detection of 13 species of foodborne pathogen in foods. J. Appl. Microbiol., 83: 727-736.
10. Manna, S. K. (2006). Detection of *Escherichia coli* O157 in foods of animal origin by culture and multiplex polymerase chain reaction. J. Food Sci. Technol., 43:77-79.
11. Louie, M.; De Azavedo, J.; Clarke, R.; Borczyk, A.; Loir, H.; Richter, M. and Brunton, J. (1994). Sequence heterogeneity of the eae gene and detection of verotoxin- producing *Escherichia coli* using serotype-specific primers. Epidemiol. Infect., 112:449–461.
12. Masoumeh, N.; Abdollah, K.; Mohammad, R.; Fatemeh, F.; Raheleh, R. A.; Mohammad, A. M.; Mana, H. J. and Zari, G. (2012). Study Prevalence of Verotoxigenic *E.coli* Isolated from Urinary Tract Infections (UTIs) in an Iranian Children Hospital. Microbiol. J., 6: 1–4.
13. Paton A. W. and Paton J.C. (1998). Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic *E. coli* hlyA, rfbO111, and rfb O157. J. Clin. Microbiol., 36: 598-602.
14. Brooks, J. T.; Sowers, E. G.; Wells, J. G.; Greene, K. D.; Griffin, P. M.; Hoekstra, M. R. and Strockbine, A. N. (2005). Non-O157 Shiga Toxin–Producing *Escherichia coli*, Infections in the United States 1983–2002. J. Infect. Dis., 192:1422–1429.
15. García, A.; Marini, R. P.; Feng, Y.; Vitsky, A.; Knox, A. K.; Taylor, S. N.; Schauer, B. D. and Fox, G. J. (2005). A naturally occurring rabbit model of enterohemorrhagic *Escherichia coli*–induced disease. J. Infect. Dis., 186:1682-1686.
16. Beutin, L.; Geier, D.; Steinruck, H.; Zimmermann, S. and Scheutz, F. (1993). Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. J. Clin. Microbiol., 31: 2483-2488.

17. Kaufmann, M.; Zweifel, C.; Blanco, M.; Blanco, J. E.; Blanco, J.; Beutin, L. and Stephan, R. (2006). *Escherichia coli* O157 and non-O157 Shiga toxin-producing *Escherichia coli* in fecal samples of finished pigs at slaughter in Switzerland. J. Food Prot., 69:260–266.

18. Rey, J.; Blace, J. C.; Blaco, M.; Mora, A.; Dahbi, G.; Alonso, J. M. and Blaco, J. (2003). Serotypic, phagotypes and virulence genes of shigotoxic producing *E.coli O157* isolated from sheep in Spain. Vet. Microbiol., 94:47-56.

19. Kudva, I. T.; Hatfield, P. G. and Hovdec, J. (1997). Characteristic of *E.coli* O157 and other shigotoxic producing E.coli serotypes isolated from sheep. J. Clin. Microbiol., 35:892-899.

إستعمال تقنية (PCR) لتشخيص جرثومة *E.coli* O157:H7 المعزوله من الأدرار في الانسان والأغنام

آلاء عامر شاكر و محمد جويد علوان

وحدة الامراض المشتركه - كلية الطب البيطري - جامعة بغداد – العراق.

E-mail: dr_alaa_alwgaa@yahoo.com

الخلاصة

الهدف الرئيسي من الدراسة هو لتحديد نسبة اصابة الجهاز البولي في الانسان والاغنام ببكتريا *E.coli* العترة O157:H7 وتشخيصها بتقنية PCR. جمعت 228 عينة إدرار من اشخاص يعانون من اصابات الجهاز البولي ومن الصغار والكبار ومن كلا الجنسين إبتداء من شهر كانون الاول2012 والى نهايه شهر اذار2013 .كذلك تم وبشكل عشوائي جمع 75 عينه إدرا ومن كلا الجنسين لأغنام تبدو طبيعية من مجزرة الشعله والرحمانيه وحقل كليه الطب البيطري في بغداد للفترة الواقعة من نهاية شهر شباط الى منتصف شهر نيسان 2013. زرعت العينات على الاوساط الروتينيه (وسط اكار الدم ووسط المكونكي اكار) وسط خاص وحضنت بدرجه 37م° لمدة 24-48 ساعه وبعدها تم اجراء الفحوصات الكيمياويه لتأكيد التشخيص ومن ثم زرعها على الاوساط الخاصه وإستعمال تقنية PCR للتشخيص النهائي. حيث ظهرت 8 عزلات من اصل 228 عينه إدرار موجبه لبكتريا *E.coli O157:H7* وبنسبة (3.50%) منها 4 في الاطفال و4 في البالغين بنسبه 2.6% في الاناث مقارنه مع الذكور بنسبه (0.87%).وعند تشخيصها بتقنيه PCR كانت 7 عزلات تمتلك eaeA جين و8 تمتلك hly A جين و2 تمتلك Stx2 جين.اما في الاغنام فكانت 21 عزله بنسبه 28% من اصل 75 عينة هي *E.coli* موجبة, 13 عزلة من اصل 75 عينة كانت *E.coli O157:H7*, بنسبة (14.6%) في الكباش و(2.6%) في النعاج , 10 و 13 و 12 و 4 و 3 تمتلك Stx2 & Stx1,hly A, hly A plasmed, eae A جين وبالتتابع عند تشخيصها بتقنية PCR. وعليه تعتبر الاغنام الميناء لسم الشيكا المنتج من قبل بكتريا *E.coli O157:H7* التي تسبب عدوى الجهاز البولي والفشل الكلوي في الانسان.

الكلمات المفتاحية:الاشريكيه القولونيه O157:H7، تفاعل البلمرة المتسلسل، التشخيص المختبري.