Pak. J. Biotechnol. 13 (4) 275 – 278 (2016)
www.pjbt.org
ISSN Print: 1812-1837
ISSN Online: 2312-7791

Short Communication

MOLECULAR DIAGNOSTIC OF ESCHERICHIA COLI AMONG URINARY TRACT INFECTIONS' PATIENTS USING POLYMERASE CHAIN REACTION (PCR)

Israa Adnan Ibraheam*, Ishraq Abdul Amir Saleh*, Hawraa Jawad Kadhim*, Zainab Khudhur Ahmad Al-Mahdi **

*College of Science for women, University of Babylon, Babil, Iraq, **College of Dentistry, University of Babylon, Babil. Iraq

Article received 12.7.2016; Revised 18.11.2016; Accepted 28.11.2016

ABSTRACT

Infection *with E. coli* represented the highest causes of Urinary tract infections among community. Molecular techniques such as polymerase chain reaction (PCR) have become most important technique for more rapid and accurate for detection bacterial causal organisms in species level. *E. coli* based on detecting gene targets *lac Z* which encode for the enzyme β-galactosidase is important target gene, this gene is responsible for energy production through break down of disaccharide lactose to the glucose and galactose. The urine samples were collected from UTI's patients initially diagnosed according to the presence of pyuria, a classical culture methods for detection of *E. coli* was recorded the presence of *E. coli* in 20 out of 30 UTI's samples (66%) while in molecular method by using polymerase chain reaction (PCR) for detection *lac Z* gene, *E. coli* was detect in 18 out of 30 UTI's samples (60%), this difference between classical culture and PCR for identification of *E. coli* was non-significant (p=0.05), and this may be due to the presence of evolved beta-galactosidase *lac Y* in some strain of *E. coli* which have the same galactose permease activity as *lac Z* galactosidase. So the present work strongly encouraged using PCR technique as a novel, perfect and fast test for *E. coli* diagnosis on molecular level. Further study for developed multiplex for both *lac Z* and *lac Y* galactosidase is highly recommended.

Key words: *lac Z*, *E. coli*, galactosidase, polymerase chain reaction (PCR).

INTRODUCTION

Escherichia coli are responsible to cause 80-85% of community urinary tract infections [1]. Urinary tract infections bacteria enter the bladder via the urethra. Urinary tract infection also can occur by blood or lymph infections. Anatomy structure of female facilitate the transmission of bacteria from the bowel to the urethra, so females are greater risk for UTI due to the anatomy following the entry of *E. coli* to the bladder, the bacteria attach the bladder wall forming biofilm that responsible for evading the immune system response against these bacteria [2]. Female are more prone to urinary tract infections than male due to the little distance between the urethra and anus [3]. Female hormone estrogen play an important role in protection against UTI bacteria, Women risk to UTI increase when women estrogen level decrease during menopause because the loss of protection of vaginal micro flora [3]. Recurrent UTI occur after vaginal atrophy after menopause [4]. *E. coli* is the commonest cause of community acquired UTIs in inpatient and out-patient settings. *Enterobacter species* is the other common cause of UTI as well as *Staphylococcus saprophyticus*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Pseudomonas species* [5]. Molecular technique such as polymerase chain reaction (PCR) has become important way for more accurate and rapid method for detection bacterial species [6, 7]. PCR method used for detecting *E. coli* in water based on detecting gene targets such as *lac Z* and lamb [8]. UTI is the most common infection in patient with indwelling catheter because bacteria are unavoidable in this patient group. *E. coli* respon -sible for 70-90% of community acquired UTI [9].

MATERIALS AND METHODS

Sample collection: Urine samples are collected from UTI patients with clean catch midstream technique. Most of patients with dysuria show urinalysis, both of bacteria and pyuria are usually improves UTI patients [9]. Pyuria can be detected by the presence of pus in urine sample under microscope and can be quantified by calculation of leukocyte count in urine samples and leukocyte can be measured using haemocytometer. Thirty samples were collected from UTI's patients from both male and females of different age groups. The samples were centrifuged and cotton swab was used for inoculation of brain heart infusion broth. The media then incubated for 3h at 37°C and the culture was saved in refrigerator for DNA extraction purpose. At the mean time second swab used for inoculation eosin methylene blue media for detection of *E. coli* in their green metallic sheen formation at this media.

Molecular Identification of Bacteria

1-DNA extraction: DNA was extracted from brain hearth infusion broth by participation of

bacteria by 7000rpm/min. and extraction using genomic DNA kit (Geneaid, China).

2. PCR amplification

PCR assay was performed to detect *Lac Z* gene which is specific for the identification of *E. coli*. By using specific primers as shown in Table-1 and these primers synthesized by Bioneer company. PCR reaction was conducted in 50µl of reaction mixture containing 25µl of green master mix, 2µl of each primer, 10µl DNA template and 11µl of deionized water as shown in Table-2. Amplification was conducted using thermocycler Ependroff programmed cycler for initial denaturation at 94°C for 3 min., 35 cycle of denaturation at 94°C for 30sec., annealing 59°C 30sec., extension 72°C 30sec, and 7min. of final extension at 72°C.

Table-1: Specific primers

Primer Name	Primer Sequence
Lac Z Forward	ATGAAAGCTGGCTACAGGAAGGCC
Lac Z Reverse	GGTTTATGCAGCAACGAGACGTCA

Table 2: The conventional mixture of PCR working solution

Volume size	*Working Solution*
11	*Water*
2	*Forward primer*
2	*Reverse primer*
10	*DNA*
25	*Master Mix*
50	*Final Volume*

PCR products were resolved by electrophoresis on 2% w/v analytical grade agarose gels (Promega, USA) stained by ethidium bromide, with the use of 100 bp DNA ladder from (Intron, Korea) visualized using UV transeliminator and documented using digital camera (Sony, Japan). and run in TBE(1X) buffer, Gels were stained with ethidium bromide (0.5 µgml^{-1}) and analyzed using UV eliminator. The molecular weight identification of resolved band was based on their correspondence to the ladder bands.

Results

The result show that the *E coli* was identified in 20 out of total thirty UTI's samples in 12 eiosin methylene blue agar which inoculated with *E. coli* for demonstrating metallic sheen growth of *E coli*, this represent 60% of total UTI's causal organisms (Fig -1), while gel electrophoresis show 18 samples out of total 30 UTI's samples have the specific *E. coli* gene *Lac Z* with clear 264 bp band size (Fig- 2).

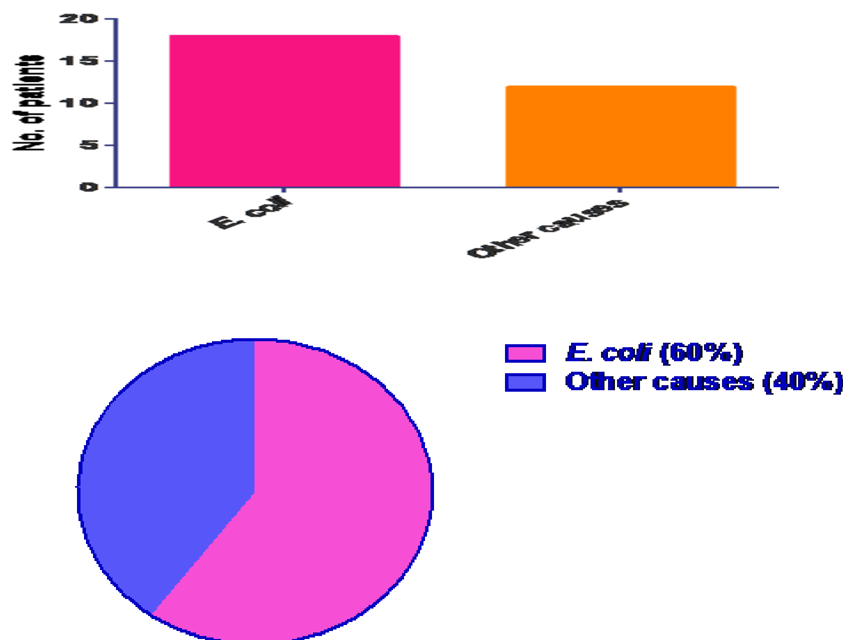

Fig.-1 The incidence of *E. coli* UTI's and other causal organisms by using PCR.

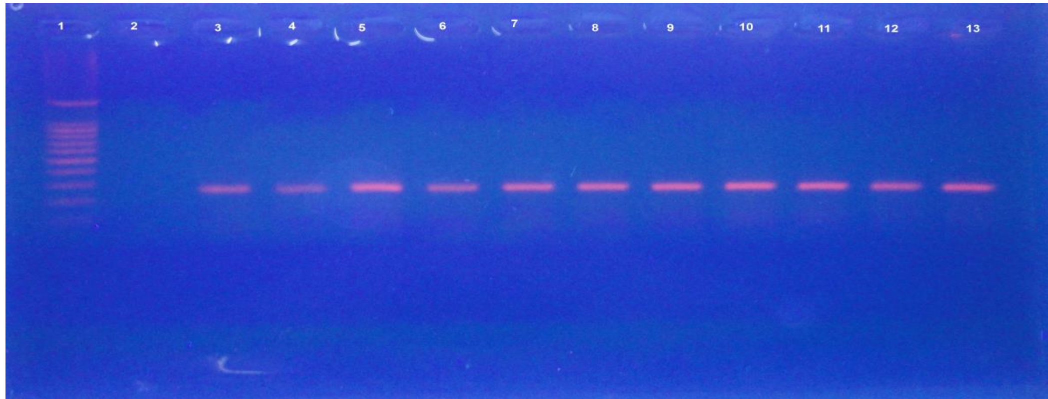

Fig.2 Agarose gel electrophoresis
Lane 1: 100 bp DNA ladder, Lane 2: negative control, Lane 3-13: positive results

DISCUSSION

E. coli represent the highest UTI's causal organisms among other causal organisms in both classical culture method (66%) and in PCR method (60%), this result was in agree with previous study in Iraq [10]. Most of the bacteria which often seen in UTI are fecal bacteria, these bacteria were mostly found in feces, while anaerobic bacteria rarely cause UTI. Most of UTI (90%) in patient with normal anatomic structure are caused by *E. coli*, 10-20% of UTI infection are caused by *Staphylococcus saprophyticus* (young sexually active females) and 5% is caused by another *Enterobacter*.

Bacterial pathogenesity: The ability of bacteria for producing fimbriae (P, S, type 1 and Dr) is important for bacteria to attach the uroepithelial cells and thereby avoid elimination. Uropathogenic *E. coli* can resist killing by complement systems. Bladder and kidney epithelial cells can internalize *E. coli* cells. Type-1 fimbriae are important in bacterial attachment to the epithelial cell in the host and promote the reorganization the epithelial cells for internalize the bacterial cells in epithelial vacuoles to enhance bacterial cells survival by providing protection from immune defense and providing bacteria for access to the deeper tissues. Once bacteria are internalized in the epithelial cells the bacteria can grow forming pod like structures. Bacteria in the epithelial cells can form a reservoir within the bladder mucus membrane and represent as agent of acute recurrent infection (20%). Result show classical culture methods for detection of *E. coli* was recorded the presence of *E. coli* in 20 out of 30 UTI's samples (66%) depending on a biochemical activity of *E. coli* on Eosin Methylene Blue Agar (EMB), which give the distinguish green metallic sheen, while molecular method by using polymerase chain reaction (PCR) for detection *lac Z* gene was detect *E. coli* in 18 out of 30 UTI's samples (60%), this differences between classical culture and PCR for identification of *E. coli* was non-significant (p= 0.05), and this may be due to the presence of evolved beta-galactosidase *lac Y* in some strain of *E. coli* which have the same galactose permease activity as *lac Z* galactosidase [11, 12]. So, the present work strongly encouraged using PCR technique as a novel, perfect and fast test for E. coli diagnosis on molecular level. Further study for developed multiplex for both *lac Z* and *lac Y* galactosidase is highly recommended. Other cause for the differences may be to the deletion mutation for the *lac Z* gene may be due to the fact that brain heart infusion broth did not contain the lactose as a source of carbon while it contains the dextrose instead of lactose [13]. *lac Z* gene may be deleted since brain heart broth did not contain lactose, the 2 control of the lac genes depends on the availability of lactose to the bacteria [14]. While tetrazolium lactose and lactose in Macconkey agar media have range 100-1000units, being most sensitive in the high and low parts of this range respecttively Since MacConkey lactose and tetrazolium lactose media both rely on the products of lactose breakdown, they require the presence of both *lac Z* and *lac Y* genes. The many lac fusion techniques which include only the *lac Z* gene are thus suited to the X-gal plates or ONPG liquid broths [15,16].

REFERENCES

1. Bhat, R.G., T.A.Katy and F.C.Place, Pediatric urinary tract infections. *Emergency Medicine Clinics of North America* **29**(3): 637–53 (2011).
2. Salvatore, S., S.Salvatore, E.Cattoni, G.Siesto, M.Serati, P.Sorice and M.Torella, Urinary tract infections in women. *European Journal of Obstetrics, Gynecology and Reproductive Biology* **156** (2): 131–6 (2011).
3. Dielubanza, E.J. and A.J.Schaeffer, Urinary tract infections in women. *The Medical clinics of North America* **95**(1): 27–41 (2011).

4. Jumpup Goldstein, I., B.Dicks, N.N.Kim and R.Hartzell, Multidisciplinary overview of vaginal atrophy and associated genitourinary symptoms in postmenopausal women. *Sexual medicine* 1(2): 44–53 (2013).
5. Watring, N.J. and J.D.Mason, Deciphering dysuria. Emerg. Med. 40(9):29-34 (2008).
6. Zainab Khudhur Ahmed Al-Mahdi,Validation the Best, Most Accurate and Reliable Assay for Detection of Typhoid. Karbala J. Med. **6**(1): 1561-1567 (2013).
7. Israa Adnan Ibraheam Al-Baghdady, Ashwak Bassim Jassim and Zainab Khudher Ahmed, Evaluation of the isolation and detection methods for salmonella spp. From egg shell contamination using multiplex PCR. Bas. J. Vet. Res. **14**(1): 282-288 (2015)
8. Khan, I.U.1., V.Gannon, R.Kent, W.Koning, D.R.Lapen, J.Miller, N.Neumann, R.Phillips, W.Robertson, E.Topp, E.van Bochove and T. A.Edge, Development of a rapid quantitative PCR assay for direct detection and quantification of culturable and non-culturable *Escherichia coli* from agriculture water sheds. Journal of Microbiological Methods **69**: 480–488 (2007).
9. Helina Rahman and Manab Deka, Detection and characterization of necrotoxin producing *Escherichia coli* (NTEC) from patients with urinary tract infection (UTI). Indian J. Med. Res. **139**(4): 632–637 (2014).
10. Asmak, K.B., L.D.Josef, H.Lawrence and M. Ronald, Detection of *E. coli* and *Shigella* ssp. In water by using the polymerase chain reaction and gene probes for Uid. Appl. and Env. Mic. **57**(4):1013-1017 (1991).
11. Althwani, A.N., A.B.Jasim and I.M.Alsaqir, Monitoring of drinking water quality in Baghdad city by using polymerase chain reaction (PCR). Journal of Babylon University Pure and Applied Sciences **3**(22):1087-1093 (2014).
12. Hall, B.G., Number of mutations required to evolve a new lactase function in Escherichia coli. *Journal of bacteriology* **129** (1): 540–3 (1997).
13. Hall, B.G., Changes in the substrate specificities of an enzyme during directed evolution of new functions. *Biochemistry* **20**(14): 4042–9 (1981).
14. Busby, S. and R.H.Ebright, Transcription activation by catabolite activator protein (CAP). *J. Mol. Biol.* **293**: 199–213 (2001).
15. Joung, J., E.Ramm and C.Pabo, A bacterial two-hybrid selection system for studying protein–DNA and protein–protein interactions. *Proc. Natl. Acad. Sci. USA* **97**(13): 7382–7 (2000).
16. Wilson, M.L. and L.Gaido, Laboratory diagnosis of urinary tract infections in adult patients. *Clin. Infect. Dis*. **38**:1150-1158 (2004).