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Detection of LT and ST Toxin genes for E.coli isolated from UTI

Mohammed I. Nader, Ismail H. Aziz, Tagreed A. Rehman

Institute of Genetic Engineering & Biotechnology

Abstract:

This study design ,study the importance of recurrence Urinary Tract infection caused by enterotoxigenic Escherichia coli (ETEC) via detect LT and ST toxin genes in E.coli samples isolated from patients with UTI. A total of 60 urinary samples were collected from patients with UTI especially from children Infected in the Central Child Hospital and Al-Yarmouk Teaching Hospital in Baghdad for the period 27/12/2011 to 7/7/2012.isolation of E. coli was performed according to the standard laboratory methods ,Agglutination test was done in the Central Health Laboratory and API 20E system was done in the Children Teaching Hospital. DNA was extracted from samples by High-Speed Plasmid Mini Kit DNA and pure yield plasmid miniprep system were used as a template for PCR reaction. Two sets of primers were used to simultaneously detect the genes encoding LT and ST by multiplex PCR assay. Out of 60 samples ,15/60 negative (25%),45/60 positive (75%), and infection in males was 6/45 (13.3%) While in females 25/45(56%). The percentage of LT gene 9/45 (20%), while none of samples were carrying ST gene. The results indicate that ETEC strains , with a relatively conserved genetic pool, are capable of causing urinary tract infection. Due to the importance of these strains in public health, it is suggested that the conventional methods used for their detection less accurate than molecular method Because of the great similarity with cholera toxin .

Keyword: *ENTEROTOXIN ,E. COLI, LT, ST*

Introduction:

Urinary tract infection (UTI) is a broad term that describes microbialcolonization of the urine and infection of the structures of the urinary tract – kidney, renal pelvis, bladder and urethra, as well as adjacent structures such as the perinephric fascia, prostate, and epididymis (1,2). urinary tract infection (UTI) is the second most common bacterial infection (3). Diarrhoea may be the presenting symptom in younger children with (UTI) (4,5). There have been alimited number of studies on the correlation between (UTI) and acute diarrhea (6,7), and it is still not clear when to investigate for (UTI) in young children presenting with diarrhea. Enteric gram-negative pathogens cause the bulk of the burden of diarrhea ,enteric fever and UTI that leads to more than three million deaths each year (8). The causes UTI include a wide range of viruses, bacteria, and parasites, among the bacterial pathogens, Escherichia coli play an important role (9). E. coli (ETEC) is also one of the most common causes of diarrhea in travelers as well as infants in developing countries. Some ETEC strains produce a heat-sensitive exo-toxin, LT which is plasmid encoding. Some other strains produce a heat-stable entero-toxin, ST which is genetically controlled by a heterogeneous group of plasmids. LT activates the adenylate cyclase, which in turn causes diarrhea by prolonged severe secretion of water and sodium chloride. In intestinal epithelial cells, ST activates guanylyl cyclase that causes fluid secretion. The strains that produce both LT and ST toxins cause severe diarrhea[10].

These E. coli strains are classified according to their pathogenic genes as well as the organ they infect . The presence of pathogenic genes specific to intestinal strains in non-intestinal strains may indicate some levels of genetic combination that have led to the creation of more invasive strains. Identification of these shared pathogenic genes can be helpful to design new diagnostic methods and plan for an appropriate program to prevent spreading of these strains in the population The aim of this study was to screen for the genes encoding enterotoxigenic Escherichia coli (ETEC) including LT and ST

Corresponding Address:
Ismail H. Aziz
Institute of Genetic Engineering & Biotechnology/ University of Baghdad
Email: iaziz66@yahoo.com

toxins among E. coli isolates from urinary tract infections.

Material and Methods:

Clinical specimens: A total of 60 urinary samples were collected from patients with urinary tract infection. The samples were collected from some private clinical laboratories located in AL-Yarmouk teaching hospital and children teaching hospital at Baghdad city during 2011/12/27to 2012/7/7.

Isolation: A loop full of urine samples were cultured on MacConkey agar by streaked and incubated for 24 h at 37 ºC, bacterial growth isolation and further identification (11).

Conventional biochemical identification of E. coli :-

Biochemical identification of E. coli isolates among bacterial isolates was done using conventional tests including Indole production, Methyl red, Hydrogen sulfide (TSI), Urea hydrolysis, D-glucose acid, D-glucose gas, Lactose fermentation, Sucrose fermentation, Dulcitol fermentation, D-mannitol fermentation, Myo-Inositol fermentation, Maltose fermentation. The identification of E. coli isolates according to[Goldman and Lorrence, 2009; Rajeshwari et al ., 2010]. (12,13).

Bacterial identification by API 20E system:- The test was carried out according to the manufacturer's manual (bioMerieux , French).

DNA extraction: plasmid DNA was extracted from cultured E. coli by using High-Speed Plasmid Mini Kit (Geneaid,Taiwan). Then the DNA was used as template for PCR.

Primer selection: The primer sequences (Bioneer,Korea) used to amplify genes, these genes are shown inTable 1

Table 1: Primers used in the multiplex PCR for amplification of Enterotoxigenic E.coli genes .

Target genes	Primers sequences (5-3)	Amplicon size (bp)	Function	Ref.
ST	GCGACAAATTATACCGTGCTCCGAATTCTGT-TATATATGT	707	Heat-labile toxin of ETEC	14
LT	GCTAATGTTGGCAATTTTTATTTCTGTAAGGAT-TACAACAAA	190	Heat-stable toxin of ETEC	NCBI*

*http://www.ncbi.nlm.nih.gov/

DNA amplification : Each multiplex PCR assay was performed in 0.2 µl eppendorf tube , each containing a total volume of 25 µl including 12.5 µl PCR master mix (Bioneer,Korea), 2µl of the extracted DNA, 1 µl for each primer . The amplification was performed in a Thermal Cycler . initial denaturation cycle 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s and elongation at 72°C for 30 s. PCR products were analyzed after electrophoresis on 1.5% agarose gel at 70 volts for1 h and stained with ethidium bromide, a molecular marke (100bpDNAladder,Bioneer,Korea) was used to determine the size of product (14).

Identification of Enterotoxigenic E.coli genes by multiplex PCR: The sample were considered negative if there is no bands were seen in the gel ,the sizes of bands on the gel was compared with marker to identify certain kinds of Enterotoxigenic E.coli genes(LT,ST for ETEC) in the urine sample. Specimens that revealed Enterotoxigenic E. coli were subjected to uniplex PCR for more conformation of the mixed infection and also for conformation of the specificity of the test.

RESULTS:

A total of 60 urinary urea specimens were collected from patients with Urinary tact infection were studied .there were 15/45 (%71.4) males and 30/45 (%76.9) females, table 2.

E.coli isolated and identified by using conventional biochemical method and API20E system , table 2. The DNA plasmid was extracted by two different Kit [high-speed plasmid mini kit (Taiwan), pure yield plasmid miniprep system (Promega,USA)] and amplified by PCR under the same conditions .

Two different genes of in order to detect Enterotoxigenic E.coli , a mixture of two primer pairs specific for target genes were used in one PCR reaction table (1) . the multiplex PCR detected targeted gene of ETEC (LT) 9/31 (%29.03) ,while none of samples was carrying ST gene Figure (1).

Table 2: E.coli identified by API 20Eand biochemical reaction

Genus	N.of patients	N.infected with *E. coli*	
		Number	Percentage
males	15	6/45	13.3%
females	30	25/45	56%
total	45	31/45	68.8%

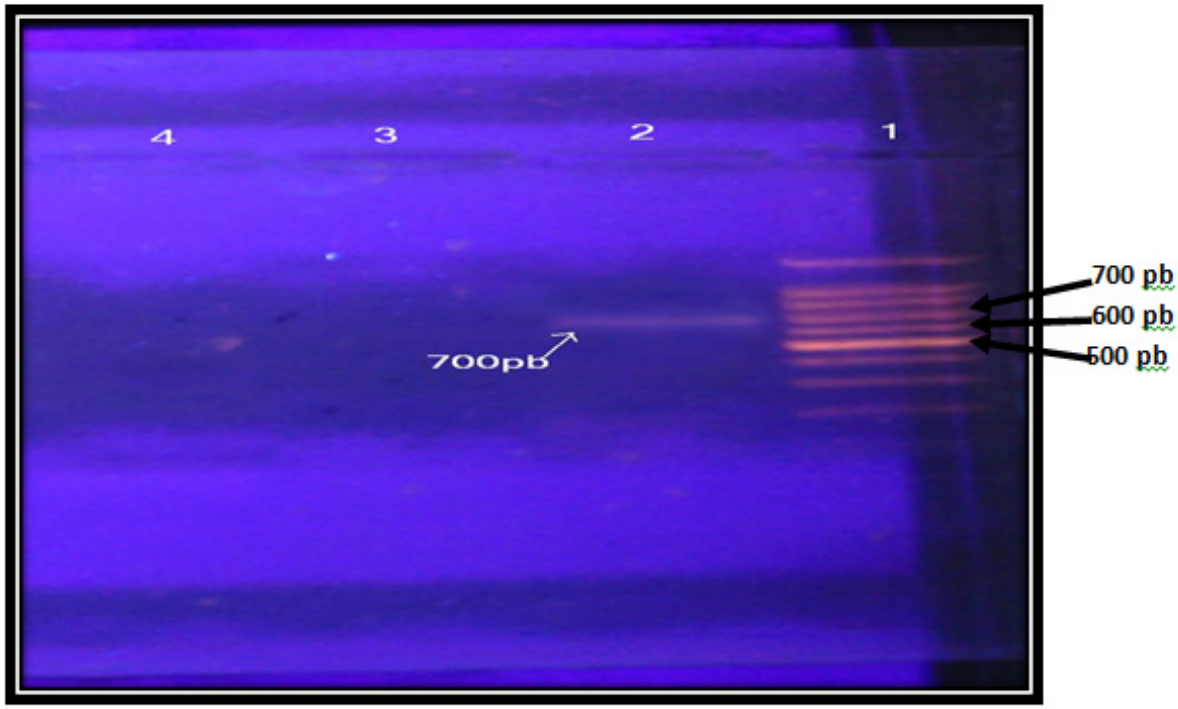

Figure1 : Multiplex PCR products electrophoresres on 1.5% agarose gel at 70 volts for1 h
Lane 1: 100pb DNA ladder
Lane 2: Sample(No22) LT gene
Lane 3: Sample(No41) negative
Lane4: Sample(No30) negative

DISCUSSION:

Urinary tract infection (UTI) is one of the most common bacterial infections in human being The infection is mainly caused by E.coli .Like other bacteria, in addition to a set of vital genes, different strains of E.coli have the ability to gain some genetic information that helps them to sustain the situation(15).Horizontal transfer of this genetic information leads to the genotypic improvement of pathogenic strains and exacerbates their pathogenicity.

Mainly, pathogenic genes located on genetic cassettes such as mobile elements, bacteriophages, and plasmids are able to be transferred to other strains. Theoretically, the phenotypic changes occur through either loss or gain of genetic information or turning on/off of the genes .As the metabolic pathways of pathogenic E. coli change or shift, the genotype of the pathogen can be changed due to selective pressure(16,17).

Presence of some pathogenic genes in different strains that infect different organs is due to either the importance of these genes in pathogenicity or their linkage with other important pathogenic genes. LT and ST toxin genes are the main pathogenic elements of ETEC strains. These strains are intestinal E. coli and cause diarrhea in infected individuals ,also can cause urinary hemolytic syndrome which often happens after an intestinal infection (18).in this study , Observed that E.coli bacteria were more causes of urinary tract infection, where

it formed rate 75% an approach the ratio obtained by the researcher (19) Where the rates of E. coli isolation of patients 78%. This result was not consistent with the search results (20) To be held in Istanbul, Turkey, where the incidence of E. coli 63%. that a high prercentage of E. coli bacteria isolated from the urinary tract of patients may be due to the presence of her naturally in the human digestive tract and move Via gastric tube – enteric to urinal canal of infected person or may be due to the hygienic status in different communities (21).

The incidence of infection in women more than men where it Formed 13.3%,56% in males and females respectively, It may be the cause of the high infection rate in females than males to anatomical differences between the sexes, so the shortness of female's urethra and Near Slot outlet that's made it easily target of inflammation , adding to existence of zinc compounds within secretions of prostate gland that could available of protection for males from infection (22,23). For urine samples that showed no bacterial growth of 25% , This may be due to the possibility of patients were taking antibiotics as a treatment, which led to the disappearance of the bacteria that cause infection Or perhaps due to the presence of other pathogens, such as anaerobic bacteria , some of Fungi such as (Trichosporon beiglii and Candida spp) and parasites (Trichomonas vaginalis , Schistosoma urethritis) (24). The percentage of LT gene 9/45 (20%) of the E. coli causing urinary infections were ETEC .Whereas, strains with no ST toxin genes. In case of toxin genes, seems that they can be expressed after some minor genetic changes and have the potential to enhance the pathogenic severity of bacterium. Therefore, it is suggested that the conventional methods for detection of ETEC strains which are based on their phenotypic characteristics LESS accurate than molecular method especially depend on the presence of LTgenes.

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الكشف عن جينات الذيفان Lt و St لبكتريا الاكولاي والمعزولة من التهاب المجاري البولية

محمد ابراهيم نادر الطائي، اسماعيل احسين عزيز، تغريد عبد الرحمن كريم

معهد الهندسة الوراثية والتقنيات الإحيائية/ جامعة بغداد

الخلاصة:

إن الهدف هو دراسة أسباب الإصابة المتكررة بالتهاب المسالك البولية نتيجة الإصابة ببكتريا enterotoxigenic Escherichia coli (ETEC) عن طريق الكشف عن جينات الذيفانات (LT ,ST) والمحمولة على البلازميدات والموجودة في بكتريا Escherichia coli والمعزولة من المرضى لمصابين بالالتهاب المسالك البولية UTI)). حيث تم جمع 60 نموذج من الإدرار من المرضى المصابين بالالتهاب المجاري البولية وبالأخص الأطفال الراقدين في مستشفى الطفل المركزي واليرموك التعليمي في بغداد وفي الفترة 2011/12/27 إلى 2012/7/7 , و أثبتت إن العزلة كانت تعود إلى بكتريا الـ E.coli بالاختبارات االمختبرية التقليدية والاختبارات المصلية التي أجريت في مختبر الصحة المركزي , واختبارات API 20E التي أجريت في مستشفى الطفل المركزي , إذ كانت 60\15 عزلة سالبة للفحص أي بنسبة 25% و 60\25 عزلة موجبة للفحص أي بنسبة 75%, وان نسبة إصابة الذكور 45\6 أي بنسبة 13,3% بينما الإناث كانت 45\25 أي بنسبة 56%.

استخلص ألـ DNA البلازميدي من عزلات E.coli باستخدام High-Speed Plasmid Mini Kit حيث استخدم بعدها كقالب في تفاعلات PCR إذ تم استخدام نوعين من البادئات وإدخالها في تفاعل PCR واحد .كانت نسبة جينات ألـ lt (9\45) 20% في حين لم يتم الحصول على جينات ألـ st .وبذلك أثبتت النتائج أن سلالة ETEC تحمل جينات الضراوة مما يجعلها قادرة على إحداث الإصابة بـ UTI(لذا تعتبر مهمة في مجال الصحة العامة) , إن الاختبارات المصلية و المختبرية التقليدية لم تعد كافية للكشف بالإضافة إلى إنها اقل دقة من الاختبارات الجزيئية في التفريق بين أنواع البكتريا وأسباب إحداث الإصابة .