WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 5, Issue 01, 143-151. Research Article ISSN 2277– 7105

POSSIBLE DETECTION OF CAG GENE IN ESCHERICHIA COLI STRAINS ISOLATED FROM PATIENTS WITH UTI

Yasmeen Reyadh Saeed*, Mohammad Sabri Abd-ALRazzaq and Mohammad Redha Joudy.

Department of Microbiology, College of Medicine, University of Babylon, Iraq.

Article Received on
10 Nov 2015,

Revised on 30 Nov 2015,
Accepted on 22 Dec 2015

***Correspondence for Author**
Yasmeen Reyadh Saeed
Department of Microbiology, College of Medicine, University of Babylon, Iraq.

ABSTRACT

In this study, (100) urine specimens obtained from patients suffering from Urinary Tract Infection and also, 25 stool samples were taken from healthy individual as control group were included, from both sexes who admitted to Al- Hilla Teaching Hospita during a period from February (2015) to May (2015). The samples were then cultured on selective media to investigate on *Escherichia coli*. Out of the 100 urine samples, only 80 samples showed positive growth and for about 40 isolates of *E.coli* were isolated where 10 (25%) of the isolates from male patient and 30 (75%) from female patients. Moroever, 7 isolates *E.coli* were isolated from stool samples of human individual. Molecular detection of cytotoxic associated gene A (*cagA*) was done to show the sequence homology of this gene in *H.pylori* to that in E.coli by using PCR technique. The results showed that all isolates of *E.coli* from urine and stool gave negative result for this marker in *E.coli* which confirm that there is no sequence homology of this gene with that present in *H. pylori*. The same results were seen with cytotoxic associated gene E (*cagE*)when specific marker from *H. pylori* is used. Besides, the isolates of *E. coli* were subjected to show their possession of *virB*II gene, by using specific marker. It was observed that 25 (62.5%) of bacterial isolates from urine and 5 (71.4%) of bacterial isolates from stool gave positive result for this gene, which is not previously detected in *E. coli*. This result indicate the presence of sequence homology of this gene with that present in *H. pylori*.

KEYWORD: Al- Hilla, *H.pylori*, *E.coli*,

1.1 INTRODUCTION

Escherichia coli is known to be one of the most common causes of urinary tract infection in the human, although it lives as commensal in human intestine.[1]. For the initiation of UTI, colonization of UPEC is required. Therefore, colonization and adhesion to host tissue is crucial for UPEC pathogenesis. At this stage, expression of the P-fimbriae freguently present in UPEC, and especially in UPEC strains isolated from patients with pyelonephritis, is extremely important.[2]

No previous studies indicate the presence or absence of cytotoxic associated genes *(cag genes)* in coliform bacteria but it is present mainly in *Helicobacter pylori* which is located within special loci in *H. pylori* genome called cag pathogenicity islands.[3]

Cag pathogenicity islands in *H.pylori* include more than 28genes, but the main genes associated with bacterial pathogenicity are *cagA*, *cagE* and *virB* genes.[4]

Cag protein is considered one of the important factor in stimulation of duodenal ulcer and also in human cancer, where *cag* gene product is associated with the events of signal transduction.[5]

So, the aim of this study to: collect of urine specimen from patients with UTI and stool samples from healthy peoples and to detect of *cag* pathogenicity islands in *E.coli* by using genetic markers. And also to Investigate on *virB*II gene by using genetic markers.

2.2. Patients and Methods

2.2.1. Patients

This study includes 100 patients suffering from urinary tract infections were admitted to one hospital Al-Hilla Surgical Teaching Hospital during the period from February (2015 to May 2015). Twenty five stool samples were collected from healthy person (as control).

2.2.2. Collection of specimens

The specimens collected for bacteriological analysis are described below. Those specimens were collected in proper ways to avoid any possible contamination.

Urine Specimen

The Specimen Were generally collected from patients suffering from UTIs**.** Mid-stream urine specimens were collected in sterilized screw-cap container andcatheter-obtained specimens

were collected. a loopful (0.01 ml) urine specimens had inoculated on the culture media (MacConkey agar, Blood agar, EMB agar) and incubated aerobically at 37C° for 24 hr according to, then Biochemical tests were processed to identify the bacteria.

Stool Specimen

Twenty five stool specimens were collected from non-hospitalized healthy in sterile container. Stool culture for E.coli was done by weighing part (1g) of the stool specimen which was diluted in 2 ml of sterile saline. A loopful stool suspension had inoculated on Mac Conkey agar and incubation at 37 C° for 24 hr.

2.2.3 Primers Sequences

The primers sequences and PCR condition that used in study are listed in table (1**)**

Table (1): The primers sequences and PCR condition

Genes	Primer sequence (5´- 3´)	Size BP	Reference	Condition
cagAF cagAR	ATAATGCTAAATTAGACAACTTGAGCG AAGAAACAAAAGCAATACGATCATTC	128	Tomasini *et al.*,[8]	95℃ 7min 1x 95℃ 0.5min 57℃ 1min 35x 72℃ 1min 72℃ 7min 1x
cagEF cagER	TTGAAAACTTCAAGGATAGGATAGAGC GCCTAGCGTAATATCACCATTACCC	508	Tomasini *et al.*,[8]	95℃ 7sec 1x 95℃ 0.5min. 65℃ 1min. 35x 72℃ 1min 72℃ 7min 1x
VirBIIF virBIIR	TTAAATCCTCTAAGGCATGCTAC GATATAAGTCGTTTTACCGCTTC	194	Tomasini *et al.*, [8]	95℃ 3min 1x 94℃ 1min 49℃ 45sec50x 72℃ 45sec 72℃ 7min 1x

RESULTS AND DISCUSSION

3.1. Isolation of *Escherichia coli*

In this study, a total 0f (100) urine specimens obtained from patients suffering from Urinary Tract Infection and also, 25 stool samples were taken from healthy individual as control group were included, from both sexes who admitted to Al- Hilla Teaching Hospital and privacy during a period from February (2015) to May (2015) in Al-Hilla province was included. All urine specimens were subjected for culturing on available media and it was out of the total of 100 samples, only 80 samples showed culture growth positive. It was found that only 40 isolate of E. coli were isolated where 10 (25%) of the isolates from male patient

and 30 (75%) from female patients. Moreover, 7 isolates of *E.coli* were isolated from stool samples of healthy individual Also, there are (20) samples gave negative growth. The interpretation of negative culture results may be either due to viral or fungal infections or the patient was taking antibiotics before doing the analysis. The results are shown in table (3-1).

Table (3-1): Prevalence of E. coli among other etiological agents associated with urinary tract infection.

Bacterial isolates	No. of isolates		Total
	Male (n= 30)	Female (n= 70)	
Escherichia coli	10	30	40
Other organisms	5	35	40
No growth	15	5	20
Total	30	70	100

The result of this study showed that *E. coli* was the predominant microorganism among the uropathogens. The incidence of this bacteria high among females are higher than males which might be due to varietyof factors, such as the close proximity of the female urethral meats to the anus, alteration in vaginal micro flora that play a critical role in encouraging vaginal colonization with coli forms which may lead to UTI, and pregnancy.

This rate when compared to other studies is correlated with the local study carried out by AL-Tememy,[6] who reported that the prevalence of *E. coli* is (60%) among UTI patient,but is higher than that mentioned by Zahera *et al*., [7] show the rate of *E.coli* was 30 % from common bacterial causing UTI.

3.2 Molecular Detection of *virB*II gene in *E.coli*

In this study, 40 isolates of *E. coli* were subjected to show their possession of *virB*II gene, which is present in *H.pylori* genome by using specific primer. The result showed that 25 (62.5%) of bacterial isolates gave the positive amplicon for this gene at molecular size (149 bp) as shown in figure (3-1), which is not previously detected in *E.coli*.

Although *virBII* is present mostly in *H.pylori* Tomacini *et al*., [8] and some strain of Sigella Hensley *et al*.,[9], the presence of it in *E.coli* isolated from UTI patients may give an indicator that the source of these isolates is from stool origin. However, this gene is present entirely in fecal isolates.

Moreover, the existence of *virB*II in *E.coli* may increase itspathogenicity and also increase its resistance to antibiotics.

This is the first result obtained about *virB*II in *E.coli*, which is present at a high rate (62.5%). Detection of *virB*II in *E.coli* at a high rate, although it's located with cag PAIs, may be related to that, this gene product can use more than one secretion system and not restricted to secretion system type IV as in cag gene.

Baron *et al*., [10] who found that type IV secretion systems (TFSS) mediate secretion or direct cell to cell transfer of virulence factors (protein or protein DNA complexes) from many Gram- negative such as *grobacterium tumefaciens, Bartonellatribocorum, Bordetella pertussis, Brucellasuis, Legionella pneumophila,* and *Rickettsia prowazekii*, into eukaryotic cells. Bacterial conjugation is also classified as a TFSS-like process mediating the spread of broad host plasmids between Gram- negative bacteria such as RP4 and R388, which carry antibiotic resistance genes.

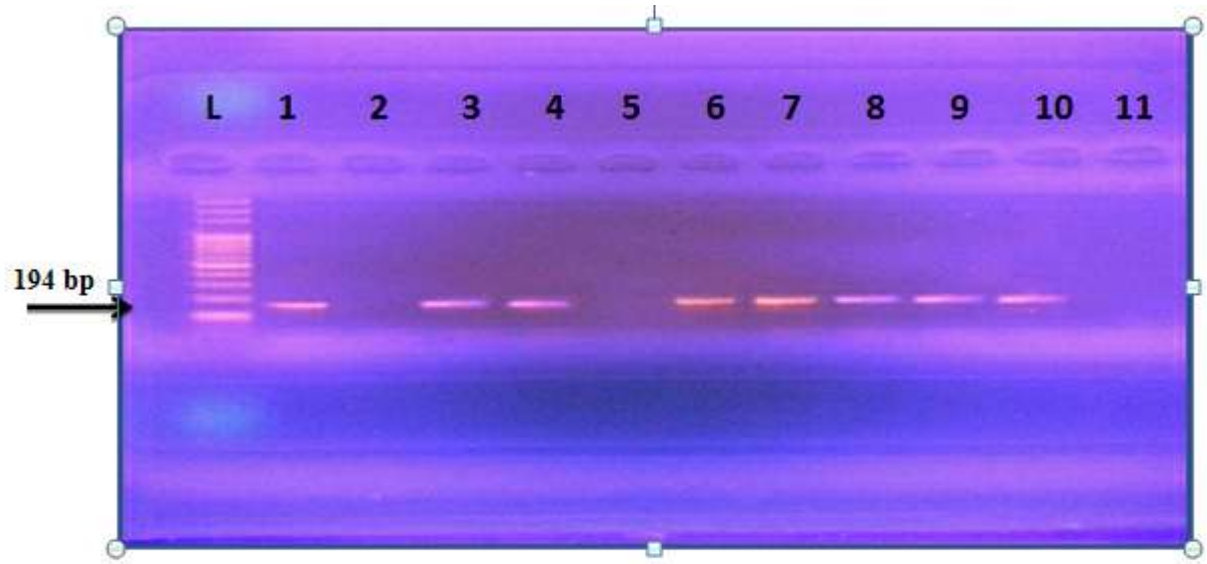

Figure (3-1) Gel electrophoresis of PCR products of *virBII* :

(1,3,4,6,7,8,9,10) isolates with positive results for *virBII* gene, (2,5,11) isolates with negative results for *virBII* gene.

L= ladder (100-1500).

3.3 Molecular Detection of Cytotoxic Associated Gene A and E (*cag A* and *E*) in *E.coli*:

In the present study, 40 isolates of *E.coli*, which have isolated from a patient with a UTI underwent for investigation of cag genes (*cagA* and *cagE*) to show the seguence homology of this gene in *H.pylori*and *E.col*i.

Besides, 7 isolates obtained from stool samples of healthy individuals are also undergone in this study.

It was shown that all *E.coli* isolates are negative for the cagA gene (with the long length in 128 bp) as shown in Figure (3-2), which confirm that there is no sequence homology of this gene with that present in *H.pylori*.

The same results obtained for the *cagE* where it is absent in *E.coli* (at molecular size 508 bp) as shown in Figure (3-2). So, the absence of cag gene pathogenicity islands in *E.coli* will ensure that this bacteria cannot induce any abnormal events in the human intestine and have no role in signal transduction where these genes are underlined to have a role in stimulation abnormalities in human intestine as in *H.pylori* where *cag* gene is considered one of the most important factors in stimulating the growth factors in human tissue.

There are many genes associated with the events of signal transduction, one of these genes is the *cag* gene.

When *E.coli* have no sequence homology to these genes present in *H.pylori*, this will under line that *E.coli* differs in their pathogenicity From *H.pylori* and it is more safe than *H.pylori* when present as a commensal.

However, although both bacteria may consider as normal flora in the human intestinal tract but the information about *H.pylori* and its role in Doudunal and intestinal carcinomas is still obscure. No previous studies had pointed out the presence of cag gene in *E.coli*.

According to this study and inspite of it being one of the most common bacteria that have multiple pathogenicity islands (PAIs) in its genome, *E.coli* was found to be free from cag PAIs and also *E.coli* has no signals in signal transduction events, and this will confirm that these bacteria have no role in cancer inducer in human.

However, Tomasini *et al.*, [8] pointed out that chromosomal cluster of cag islands may have different compositions in the infection strains of *H.pylori* and this differences may give special characteristics to these genes that be specific for *H.pylori* not for others, *cagA* and *cagE* is used widely for genotyping of H. pylori because of their heterogeneity among human strains.

The absence of *cag A* and *cag E* from *E.coli* may be attributed to that *cag A* and *E* proteins are secreted by typIV secretion system and this analogous to enteropathogenic E. coli, which translocates these like proteins via type III secretion systems Kenny *et al*., [11]

However, Mohammed and Abdullah,[3] found that only (30%) of *H.pylori* isolates gave positive results for *cagA* gene by PCR technique. Also Correa *et al*., [12] pointed out that the *cagA* gene was detected in the faecal and biopsy samples in (60%) of patients.

In another study Diab*et al*., [13] who showed that (30.2%) of *H.pylori* carry *cagA* gene and found that (18.4%) of this bacteria carry *cagE* gene. Kauser *et al*., [14] found the percentage of PCRs positive for the *cagE* genes was more than (82%) in Helicobacter pylori.
This will confirm that cag genes may be absent or mutated in some *H.pylori* strains.

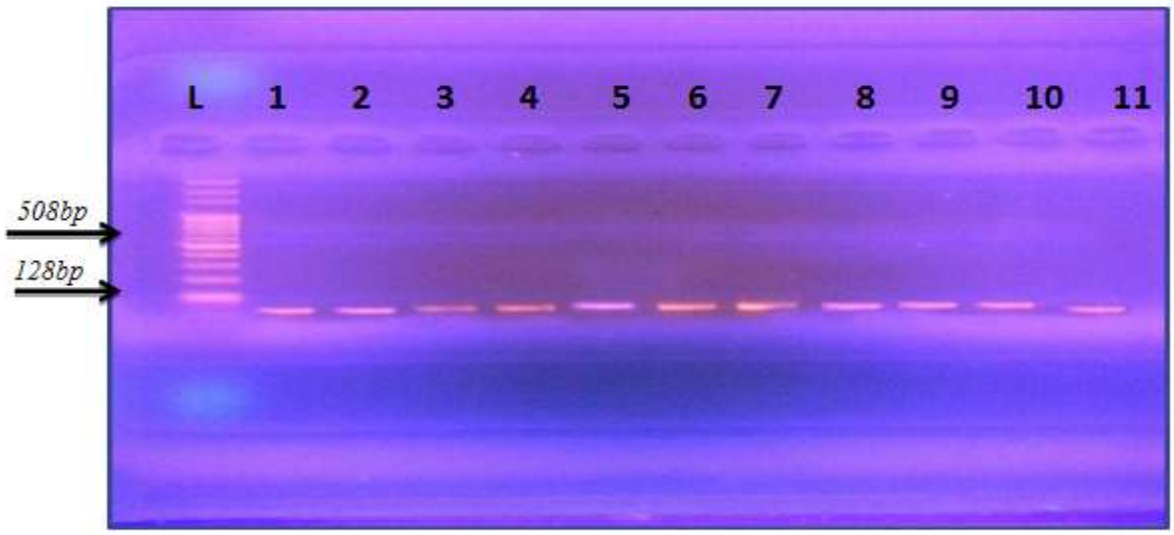

Figure (3-2) Gel electrophoresis of PCR product of *cag*A gene and *cag*E gene:

(1,2,3,4,5,6) isolate with negative results for *cagA* gene and (7,8,9,10,11) isolate with negative results for *cagE* gene.

L= ladder (100-1500).

REFERENCE

1. Azpiroz, M. F., Poey, M. E. and Laviña, M. Microcins and urovirulence in Escherichia coli. Microb. Pathog., 2009; 47: 274-280.
2. GİRAY, B., UÇAR, F. B. and AYDEMİR, S. Ş. Characterization of uropathogenic Escherichia coli strains obtained from urology outpatient clinic of Ege Medical Faculty in İzmir. Turk J Med Sci., 2012; 42(1): 1328-1337.

3. Mohammed, M.J. and Abdullah, E.M. Detection of H.pyloricagA gene in patientwithgastroduodenal disease. J. univer. of anbar for pure sci., 2013; 7(1).
4. Bourzac, K. M., Satkamp, L. A. and Guillemin, K. The Helicobacter pylori cag Pathogenicity Island Protein CagN Is a Bacterial Membrane-Associated Protein That Is Processed at Its C Terminus. InfectImmun., 2006; 74(5): 2537-2543.
5. Stein, M., Ruggiero, P., Rappuoli, R. and Bagnoli, F. 2013. Helicobacter pylori CagA: from pathogenic mechanisms to its use as an anti-cancer. Front. Immunol.
6. AL-Tememy, A. H. (2014). Dissemination of IRs of Escherichia coli isolated from patients with Urinary Tract Infection in Hilla City. M. Sc. Thesis. College of Medicine, University of Babylon. Iraq.
7. Zahera, M., Rastogi, C., Singh, P., Iram, S., Khalid, S., and Kushwaha, A. Isolation, Identification and Characterization of Escherichia coli from Urine Samples and their Antibiotic Sensitivity Pattern. Eur. J. Exp. Biology, 2011; 1(2): 118-124.
8. Tomasini, M.L., Zanussi, S., Sozzi, M., Tedeschi, R., Basaglia, G., and Paoli, P.D. Heterogeneity of cag Genotypes in Helicobacterpylori Isolates from Human Biopsy Specimens. J. Clin. Microbiol., 2003; 41(3): 976-980.
9. Hensly, C.T., Kamneva, O.K., Levy, K.M., Labahn, S.K., Africa, L.A., and Wing, H.J. Two promoters and two translation start sites control the expression of the Shigellaflexneri outer membrane protease IcsP. Arich. Microbial. Author manuscript. 2011; 193(4): 263-274.
10. Baron, C., Callaghan, D. O. and Lanka, E. Bacterial secrets of secretion: EuroConference on the biology of type IV secretion processes. Mol. Microbiol., 2002; 43(5): 1359-1365.
11. Kenny, B., Devinney, R., Reinscheid, D. J., Frey, E. A. and Finaly, B. B. EnteropathogenicE. coli (EPEC) transfer its receptor for intimate adherence into mammalian cells. Cell, 1997; 91: 511-520.
12. Correa, P., Bravo, L.E., Peek, R. M., Wilson, K. T., Sicinschi, L. A., Loh, J. T., Yepez, M. C., Gold, B. D., Thompson, D. T., Cover, T. L. and Schneider, B. G. Non-invasive Genotyping of Helicobacter pyloricagA, vacA, and hopQ from Asymptomatic Children. Helicobacter, 2012; 17(2): 96-106.
13. Diab, M., El-Dine, S.S., Aboul-Fadl, L., Shemis, M., Omran, Z., Badawi, A., El-Gannam, M., Ray, A., Fam, N., El-Defrawy, I., El-Sherbini, E., and Saber, M. Helicobacter pylori cag Pathogenicity Island Genes Among Dyspeptic Patients with Chronic Gastritis. Egyptian J. Med. Microbiol., 2009; 18(1).

14. Kauser, F., Khan A. A., Hussain, A. M., Ian M. Carroll, Naheed Ahmad, SantoshTiwari, YogeshShouche, Bimal Das, MahfoozAlam, S. Mahaboob Ali, C. M. Habibullah, Rafaela Sierra, Francis Megraud, Leonardo A. Sechi and Niyaz Ahmed. The cag Pathogenicity Island of Helicobacter pylori Is Disrupted in the Majority of Patient Isolates from Different Human Populations. J. Clin. Microbiol, November, 2004; 42(11): 5302-5308.