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Studying the Biofilm Production by *E. coli* and Their Virulence Gene in Pregnant Women with Urinary Tractinfection

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Abstract

Infection of the urinary tract (UTI) is prevalent during pregnancy and can be related to harmful effects for both the mother and the child. The elevated risk of disease in these patients was due to physiological changes, with less focus on Escherichia coli the most frequent causative factor. Our aim was to investigate the biofilm production by E. coli and Lux gene in pregnant Iraqi women with UTI. During the period extended from January to July 2020,a total of 40 from patients' samples were collected from Baghdad Teaching Hospital diagnosis by biochemical test. The results show Out of the forty samples, 14 isolates showed a sharp band on gel electrophoresis after the amplification process by PCR which mean that those 14 isolates possess the LUX gene. Those 14 samples showed higher level of biofilm production when compared to the production of biofilm by the LUX negative isolates.

Key words:*UTI*,Biofilm,*E. coli*,luxS gene,PCR- Sequencing.

Introduction

The prevalent type of bacterial infection among pregnant women is urinary tract infection (UTI) the most commonly isolated microorganism is *Escherichia coli (E. coli)* causes as much as 90% of UTIs (EARS-NET,2017).In pregnant women, hormonal and physiological variations in the urinary tract, particularly ureteral dilatation and changes in the volume of the bladder, may promote infection (Nowicki et al.,2002). Incidentally, women who have had childhood infections have a higher prevalence of UTI during pregnancy (Ramos et al.,2012). In addition, pregnant women have been shown to be vulnerable to developing persistent UTIs (Nowicki et al.,2002).

Various microorganisms, including Escherichia coli, bind to each other to form a colonizing instrument that contributes to biofilm formation (Dang and Lovell, 2016). In these groups,

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microorganisms are not protected against different environmental factors, such as availability of nutrients, temperature, pH, etc. stimulating resistance to antibiotics is one of the key benefits for biofilm growth (Soto, 2013). In addition, biofilms defend microorganisms from environmental stress and are more resistant to antimicrobial treatments and disinfectants(Bridier et al., 2015). A critical issue in the treatment of chronic infections is the decreased susceptibility of biofilms to antibiotics. Around 65% of bacterial infections are related with biofilms (Römling & Balsalobre, 2012).

Methodology

Subjects

Forty urine samples from pregnant women with UTI caused by E. coli were collected from Baghdad TeachingHospital, were examined between January to July 2020 for the presence of bacteria the ages ranging from 20 to 38 years. Then different microbiological and biochemical tests weredone to isolate a single colony of *E. coli*.

Molecular analysis

The nucleic acids were extracted by using Quick-DNA Fungal/Bacterial Micro Prep Kit (Cat, NO. D6007). Polymerase chain reaction were done in order to amplify specific region of Lux gene by using specific set primers; forward; TCCTGCAACGAGTGCATCTG, reverse;CTGCGTGCCGAACAAAGAAG(this study).The additives were done as follow 10µl of master mix 0.5 µl of each primer forward and reverse, 4 µl of eluted DNA and the distilled water.

Table (1): The PCR program used for the study.

Phase	Tm (°C)	Time	No. of cycle
Initial Denaturation	95°C	5 min.	1 cycle
Denaturation -2	95°C	10sec	35 cycle
Annealing	57°C	15sec	
Extension-1	72°C	20sec	
Extension -2	72°C	7 min.	1 cycle

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Biofilm production evaluation

The microorganism was cultivated overnight in the blood heart infusion in order to analyze the creation from biofilm. And then synchronized to 2.5 units of MacFarland, then 1/250 in the medium of BHI diluted. The plate wells (200 µL) for each test have been inoculated from the diluted suspensions.A biofilm-forming E. Coli was included as the positive control. Although only culture medium was applied to the negative controls in each experiment. Every process checked all strains in at least 3 independent experiments (Hegarty et al., 2016).

Results and Discussion

The polymerase chain reaction diagnostic techniques are rapid, easy, inexpensive protocol becoming the most commonly utilized of all molecular genetics ways for detecting important genes and identifying the bacteria PCR products of 294 bp for LUX gene were detected in the positive and PCR product was not seen in the negative samples as seen in figure (1).

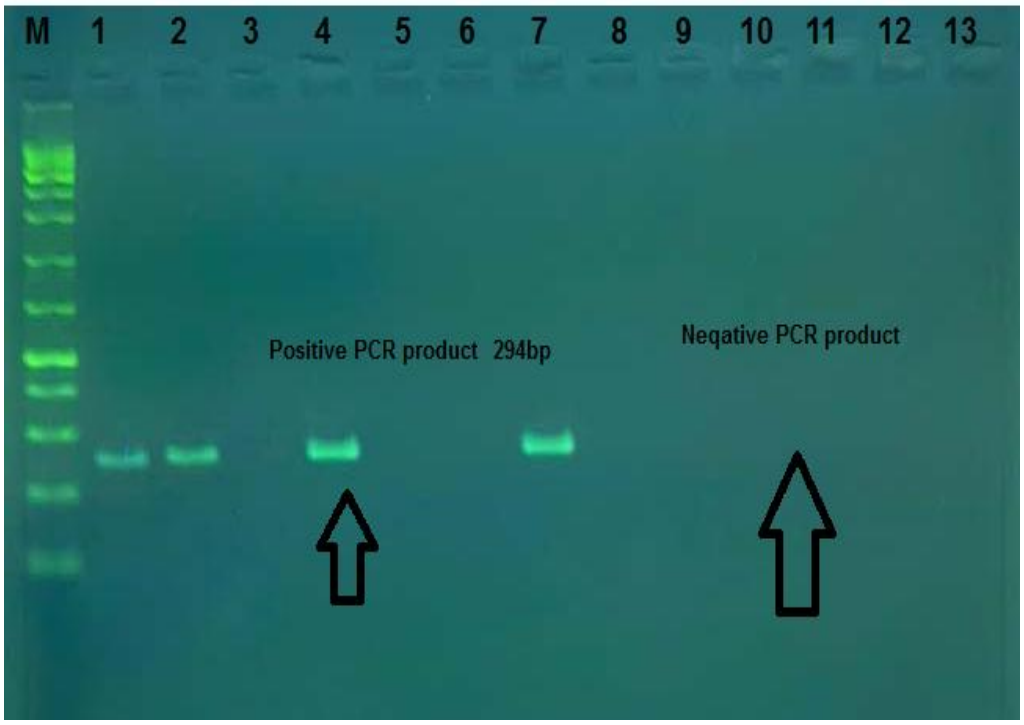

Figure (1): -amplified region of LUX gene on agarose gel electrophoresis (294bp). Bands were fractionated by electrophoresis on a 1.5% agarose gel (2 h., 5V/cm, 1X TBE) Lane: M (M: 1000bp ladder),Lane: 3,5,6,8,9,10,11,12,13(negative PCR product).

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Out of the forty samples, 14 isolates showed a sharp band on gel electrophoresis after the amplification process by PCR which mean that those 14 isolates possess the LUX gene. Those 14 samples showed higher level of biofilm production when compared to the production of biofilm by the LUX negative isolates. The results summarized in figure (2).

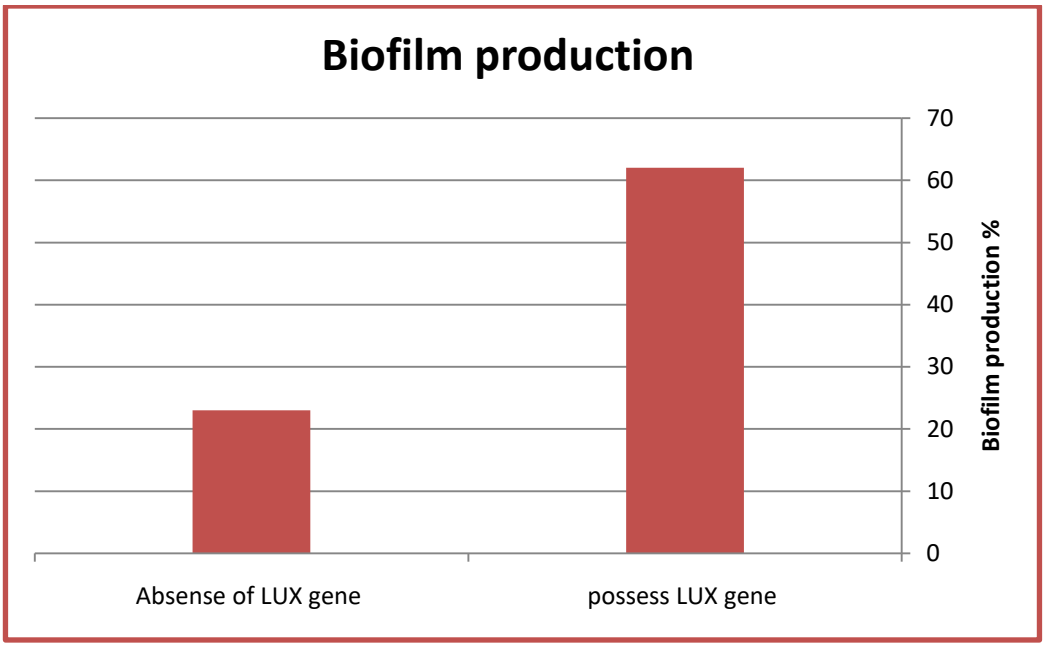

Figure (2):Comparison of the biofilm production between the isolates possess the LUX gene and the samples that don't possess the LUX gene.

The LUX gene were amplified by PCR method, and sent for sequencing service to Macrogen company Korea. The sequencing result of LUXgene shows for isolates 14, 15 from Escherichia coli of pregnant women with UTI having one TransitionT>G in locations (54 nucleotide) code GTT>GGC of amino acid VAL > GLY and Predicted EffectMissense, from the Gene Bank, found that part of LUX gene having 99% compatibility with subject of LUX gene in NCBI as seen in table (1).

Table (1): Represent type of polymorphism of LUX gene.

No. Of Isolate	**Type of substitution**	**Location**	**Nucleotide**	**Nucleotide change**	**Amino acid change**	**Predicted effect**	**Sequence ID**	**Score**	**Identities**
14,15	**Transvertion**	**54**	**T>G**	**GTT>GGC**	**VAL > GLY**	**Missense**	**CP062967.1**	**525**	**99%**

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While the analysis of the LUX gene for isolates 10, 11 from Escherichia coli of pregnant women with UTI our study was coordinated by 99% having one Transition of A>G, code AAT>TGG and amino acid transformation ASN> TRP in location (24 nucleotide), under sequence ICP062967.1, also one Transvertion T>G in locations (54nucleotide) code GTT>GGC of amino acid VAL >GLY that effect Missense as shown in table (2).

Table (2): Represent type of polymorphism of LUX gene.

No. Of Isolate	Type of substitution	Location	Nucleotide	Nucleotide change	Amino acid change	Predicted effect	Sequence ID	Score	Identities
8,10,11	Transition	**24**	**A>G**	**AAT>TGG**	**ASN> TRP**	Missense	ICP062967.1	**525**	**99%**
	Transvertion	**54**	**T>G**	**GTT>GGC**	**VAL > GLY**	**Missense**			

Compatibility of 98 percent in Gene Bank of LUX gene shows for isolate 4 from *Escherichia coli* of pregnant women with UTI having one Transition of A>G , code AAT>TGG and amino acid transformation ASN> TRP in location (24 nucleotide), under sequence ICP062967.1, as well two Transversion T>G in location (54nucleotide), the code GTT>GGC amino acid change VAL > GLY , another location T>A (110 nucleotide), have code GTC>TAC and amino acid VAL > TYR the effect Missense substitution respectively.

Table (3): Represent type of polymorphism of LUX gene.

No. Of Isolate	Type of substitution	Location	Nucleotide	Nucleotide change	Amino acid change	Predicted effect	Sequence ID	Score	Identities
4	**Transition**	**24**	**A>G**	**AAT>TGG**	**ASN> TRP**	Missense	CP062967.1	**525**	**95%**
	Transvertion	**54**	**T>G**	**GTT>GGC**	**VAL > GLY**	Missense			
	Transvertion	**110**	**T>A**	**GTC>TAC**	**VAL > TYR**	**Missense**			

The phylogenetic tree diagrammatic by Molecular Evolutionary Genetics Analysis (MEGA) software version 6.0 is shown in Figure (1). This alignment appeared the *E. coli* between Iraq and

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others in the world by Specific region within LUX gene for translating specific region. Hierarchical cluster analysis determines the following clusters including *E. coli* which was isolated from Iraqi the isolates 10,14 the same genetic dimension (1.3), while isolates 4,8,11 the same genetic dimension (1.1) all isolatesgenetic dimension (0.6) are close to *Escherichia coli* isolate MSBI16C-sc2280315 thegenetic dimension (0.7).

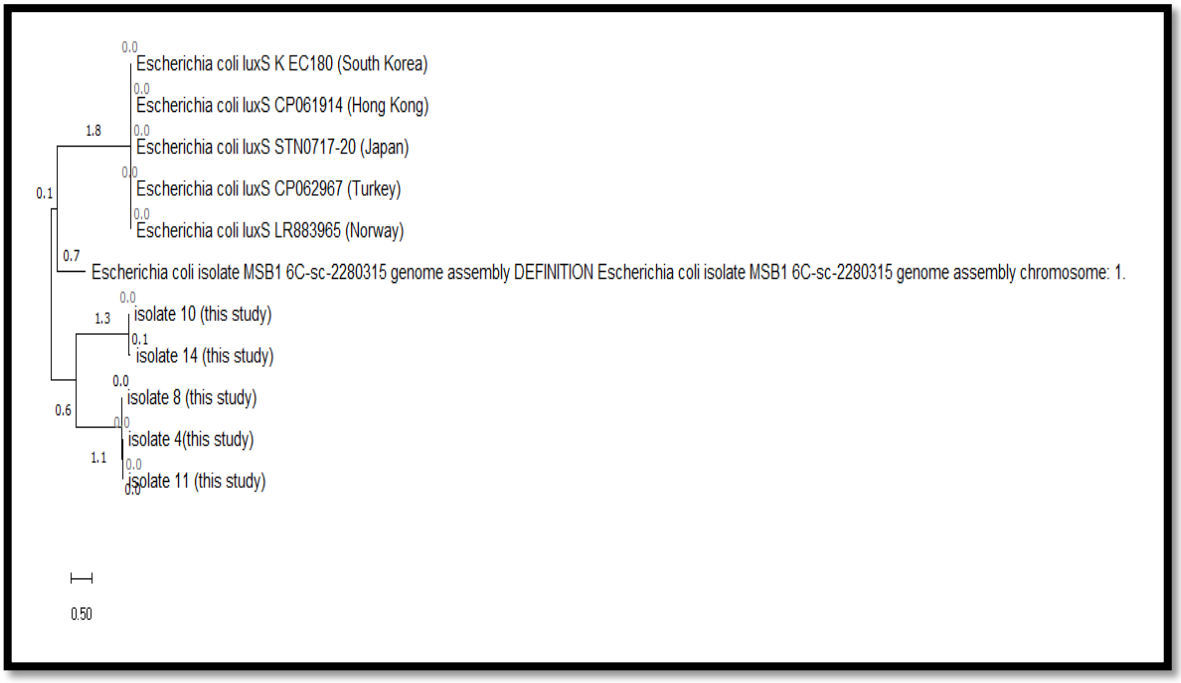

Figure (1):Neighbor-joining tree *Escherichia coli*of *LUX gene*.

E. Coli is the most common UTI causative factor in both outpatient clinics and inpatients. UTIs can culminate in serious consequences, like renal failure, if left untreated. Pyelonephritis typically occurs after a simple inflammation of the bladder (i.e. cystitis) (Bahalo *et al.*, 2013).

The capacity of pathogenic bacteria to bind to the urinary tract epithelium utilizing pili (fimbriae) is the most significant pathogenic characteristic of UTIs Various genes that encode pili can be identified by molecular techniques such as PCR (Yazdi et al.,2018).

Simple and a direct method of detection were find after the LUX gene a number for Gram-negative bacteria have been expressed. This is first study of the biofilm production by E. coli and Lux gene in pregnant women with UTI. a previous study conducted by Marincs who transferred the lux gene utilizing the plasmid vector pLITE27 to form a clinical isolate from Escherichia coli. This plasmid contains a 7 kb EcoRI fragment containing Xenorhabdus luminescens luxCDABE operon

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cloned into pUC18, enabling transcription of the lux genes from the lac promoter for pUC18.

Another study by Salisburythat done on Escherichia coli isolate that possess luxgene and this gene used by real-time which can as monitor moxifloxacin effects on bacterial metabolism compared with effects on cell replication, and the results showed metabolic activity over 54 h, in addition the greater inhibition at 1× MIC than with higher MIC multiples. And also the bioluminescence showed that post-antibiotic effect was longer when its tested by RT-PCR than by viable counts. The efficient regrowth time associated with regulation was consistent with both ways (Salisbury *et al.*,1999).

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