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Article

Pregnant Iraqi women's exposure to certain efflux pump genes of highly pathogenic uropathogenic Escherichia coli was studied molecularly.

Zena Nema Salomi[1],* , Zainab H. Abood 2
Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University Of Baghdad, Iraq.
* Correspondence: mailto:zeenanema@gmail.com
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Abstract

One hundred midstream urine samples were collected from pregnant women aged less than 20 to over 36 years suffering symptoms referred to as urinary tract infections (UTIs) from a private laboratory in Baghdad city. 90 of the urine samples gave a positive culture, and 10 gave a hostile culture. According to microscopic examination, cultural characteristics and biochemical tests, Escherichia coli was identified as the most causative agent 53(58.9%) that causing UTIs among all bacterial isolates, which confirmed the diagnosis by Vital Index of Traditional Environmental Knowledge (VITEK 2) systems and at final genetically by diagnostic gene(UidA) gene. Genetic study including extraction of chromosomal DNA from 25 E.coli isolates which have multidrug resistance (MDR) against different antibiotics classes, then identification by UidA gene and detection of acrA,acrB genes that encoding for efflux pump proteins to all 25 E.coli isolates by conventional polymerase chain reaction (PCR) amplification technique with specific primer for each gene. The results showed the UidA gene identification found in all E.coli isolates, and the result of detection efflux pump genes showed that acrA and acrB genes were present together in all E.coli isolates. The results of DNA sequence analysis of E3 bacterial isolate showed that the diagnostic genes UidA and, acrA, acrB gene were 99% belonging to E. coli as found in the National Center for Biotechnology Information (NCBI).

Keywords: Pregnant women, Urinary tract infection, *E. coli*,

Introduction

The most commonly living microorganism of the human gastrointestinal tract and also the most common causative agent of bacterial urinary tract infection is E.coli[1]. UTIs are mostly the second most common bacterial infections after respiratory tract infections seen in primary care. UTIs are an inflammatory response of the urothelium to bacterial invasion that is usually associated with bacteriuria and pyuria[2]**.** Uropathogens are the predominant type of bacterial infection among pregnant women. Uropathogens involve both Gram-negative

(Escherichia coli, Klebsiella pneumonia, K. oxytocin, Proteus mirabilis, and Pseudomonas aeruginosa) and Gram-positive bacteria (Staphylococcus saprophyticus and Enterococcus facecalis) among the joint causative agent associated with UTIs development, had been focused on Uropathogenic Escherichia coli (UPEC) that are predominantly and most frequent causative agent; it is responsible for (80 –90) % of infection during pregnancy[3,4,5]**.** E. coli is characterized by its ability of multidrug resistance (MDR)[6]**.** The pathogenic E. coli species comprise a very versatile group with numerous virulence determinants (virulence factors), including adhesions, invasions, toxins and secretion systems that allow them to act as causative agents in both human and veterinary medicine[7] and are characterized by high resistance to antibiotics as a result of possessing resistance enzymes such as β- lactamases that enhance resistance to betalactams. It also has other mechanisms that increase its resistance to antibiotics such as change of cell membrane permeability, change in the target site, inhibition of protein synthesis and bacterial ownership of pumps efflux pumps promote bacteria resistance to antibiotics such as macrolides and antidotes novobiocin and rifamicn[8]. Bacterial multidrug efflux systems are a significant and common mechanism of intrinsic antimicrobial resistance employed by bacteria efflux systems that can extrude a variety of structurally diverse antimicrobials and some metabolites[9]. The flow pumps are divided into five families. Major Family Facilitator Super Family (MFS), Small Multidrug family resistance family (SMR), Multidrug and Toxic Efflux Family (MATE), Family ATP-Binding Cassette Family (ABC) and Resistance - Nodulation - Division (RND) Family. Resistance - Nodulation - Division Family including AcrAB-ToIC, which is most common in E. coli bacteria, consists of three proteins: the inner membrane protein AcrB, which is encoded by the gene acrB and proteins Scattered in the plasma vacuum acrA encoded by the acrA gene and the ToIC channel located in outer membrane**[10]**.

Materials and Methods

Bacterial isolates collection:

53 isolates of each E. coli isolated from pregnant women having urinary tract infections admitted to private clinics. After microscopic examination, cultural characteristics and biochemical test, Isolates were 99.9% E. coli when tested by the Vitek 2 automated system.

Genotyping assay

DNA extraction:

Wizards kit was used to extract bacterial DNA.

Polymerase chain reaction process:

The diagnostic gene (UidA) and two virulent genes (acrA,acrB)were used in this study. The size and annealing time of them are mentioned in Table 1. Gel electrophoresis was done using 1% agarose at 70cm/V for 120 min.

NO.	Primer name	Sequence(5- - 3-)		Product(bp)	TM	reference
1	*uidA*	F	CATTACGGCAAAGTGTGGGTCAAT	658	40 Sec.	(11)
		R	CCATCAGCACGTTATCGAATCCTT			
2	*acr A*	F	CTCTCAGGCAGCTTAGCCCTAA	107	40 sec.	(12)
		R	TGCAGAGGTTCAGTTTTGACTGTT			
3	*acr B*	F	GGTCGATTCCGTTCTCCGTTA	107	57 Sec.	(12)
		R	CTACCTGGAAGTAAACGCATTGGT			

Table 1. E. coli's diagnostic and virulent genes.

DNA sequencing:

The Uid A, acrA, and acrB genes were amplified by PCR and sent to Macrogen Company Korea for sequencing service.

Result

Polymerase chain reaction assay:

Twenty-five isolates of E.coli with multi-antibiotic resistance were selected, and the diagnostic gene(UidA) at 658bp (figure 1) and virulence genes(acrA,acrB) of efflux pumpsat 107 bp (figure 2),(figure 3)were detected.

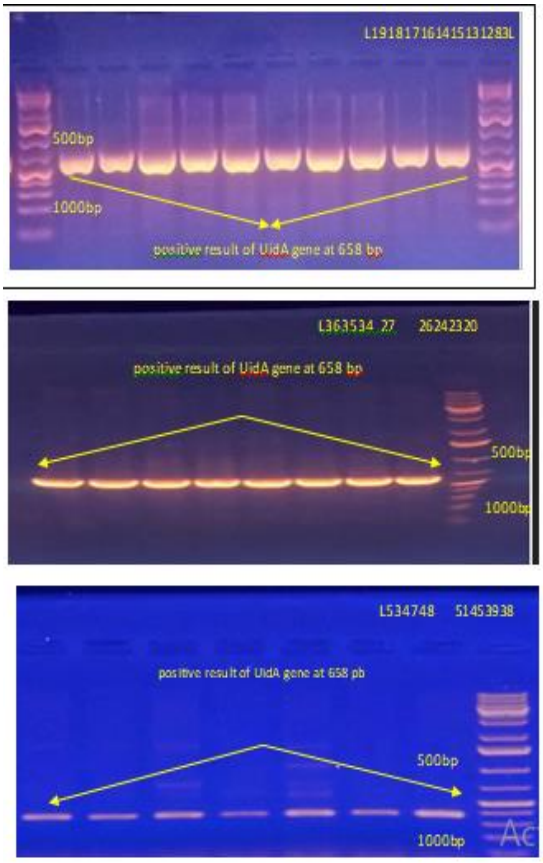

Figure 1. *E. coli* isolates had *the UidA* gene at 658bp. DNA ladder was presented as L. Gel electrophoresis was done using 1.6% agarose at 70cm/V for 120min

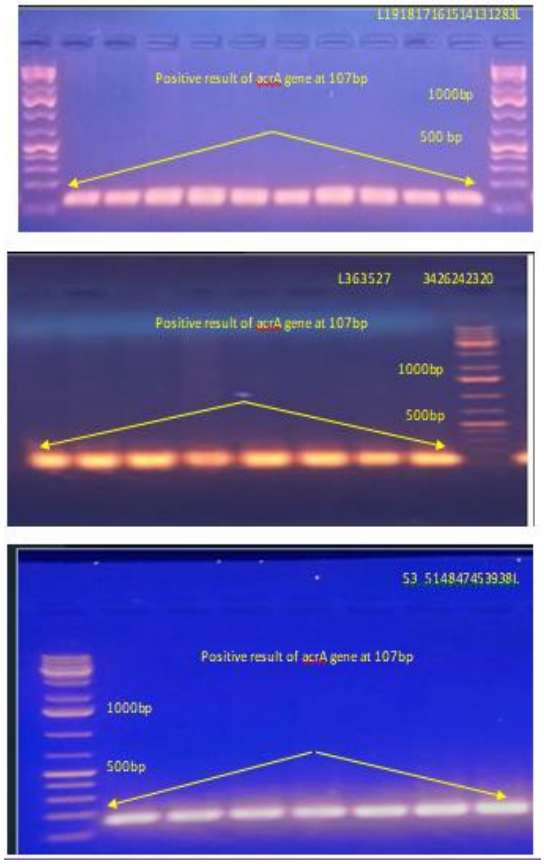

Figure 2. *E. coli* isolates had *the acrA* gene at 107bp. DNA ladder was presented as L. Gel electrophoresis was done using 1 % of agarose at 70cm/V for 120min

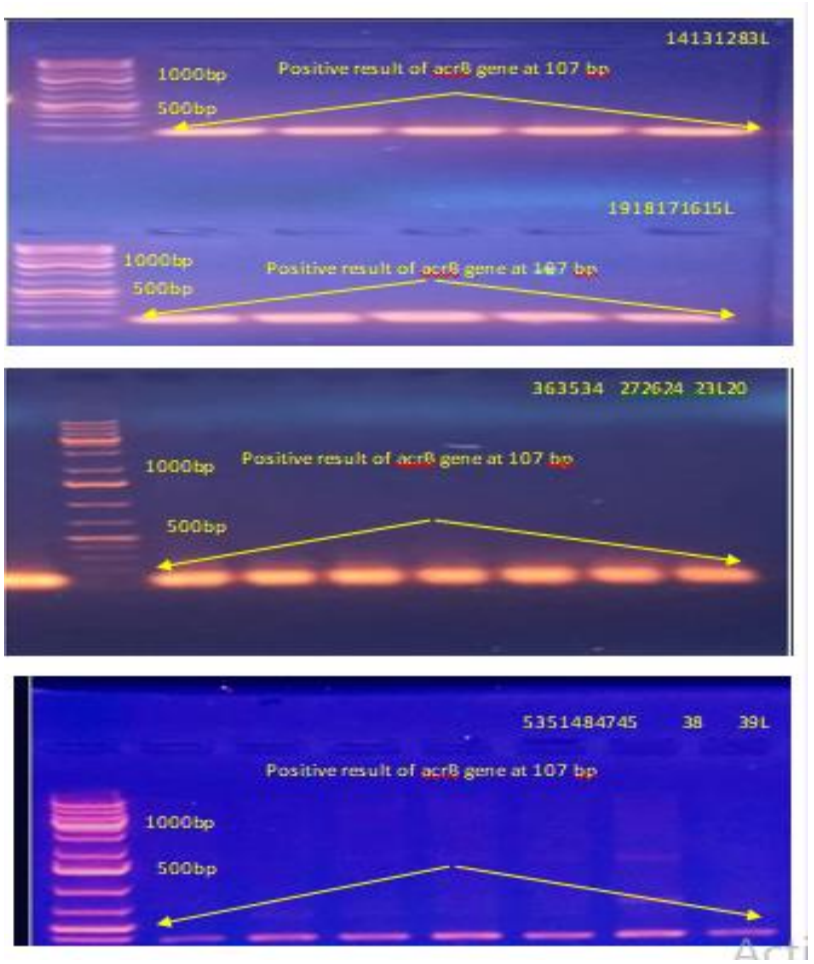

Figure 3. *E. coli* isolates had *acrB* gene at at 107bp. DNA ladder was presented as L. Gel electrophoresis was done using 1 % of agarose at 70cm/V for 120min

DNA sequencing assay:- The sequencing result of *the Uid A* gene shows that E.coli has one Transvertion G> T code GTG\GTT of amino acide Valine> Valine and predicted effect Silent, as well as one TransitionT>C code ACT\ACC amino acid change Threonine>Threonine the effect Silentas seen in Figure 4.

Part of the Uid A gene, as shown in Figure (5), has one TransvertionT\G in code GTG\GGG amino acide Valine\Glycine and missense substitution, as well one Transition C\Tin code CAA\TTA amino acid glutamine\Leucine the predicted effect missense. And Two TransvertionA\T, T\G in code CAA\TTA, GTG\GGG aminoacid Glutamine\Leucine, Valine\Glycine the missence substituation.

While sequencing for *acrA* gene to E.coli having one TransvertionG\T in code GTG\GTT for amino acide Valine\ Valine, the effect is Silent . as seen in figure (6). The sequence analysis of the *acrB* gene for E.coli, as seen in figure 7. having one Transition C\T in code GTG\ATG amino acideValine\Methionine the predicted effect Missense, as well as one oneTransvertion T\G in code AAA\ACA amino acide Lysine\Threonine the effect Missense.

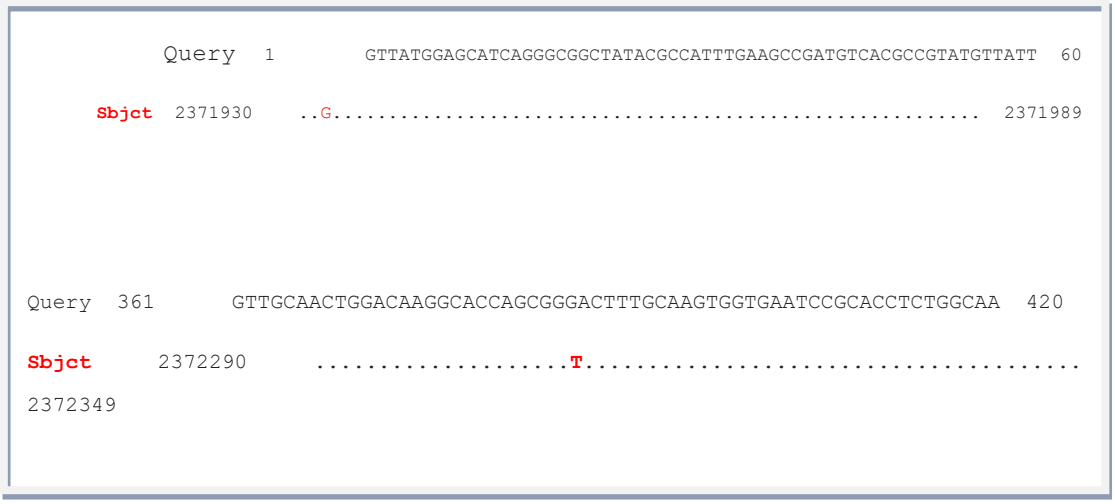

Figure 4. Sequence analysis of *UidA* gene of *E.coli* with Gene Bank of NCBI. Quray represents the sample; the Subject represents a National Center Biotechnology Information (NCBI) database.

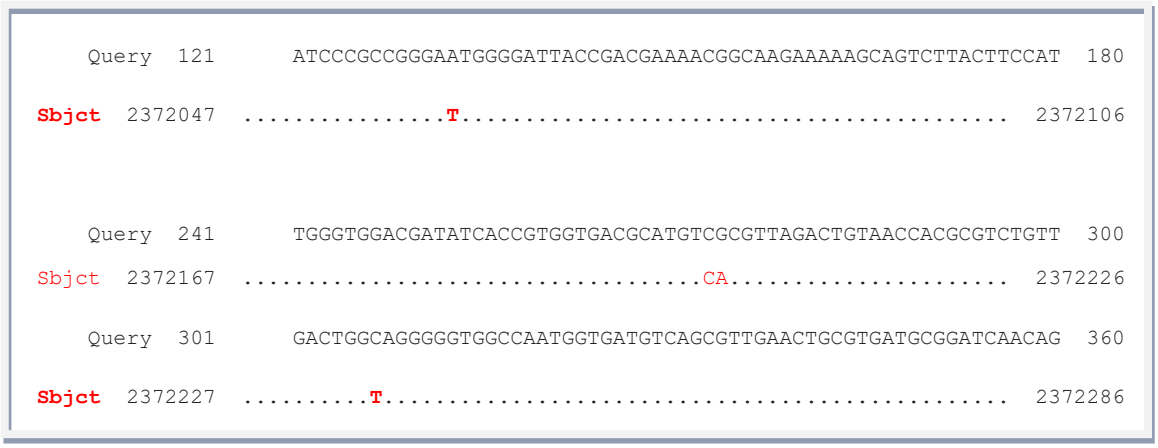

Figure 5. Sequence analysis of *UidA* gene of *E.coli* with Gene Bank of NCBI. Quray represents the sample; the Subject represents a National Center Biotechnology Information (NCBI) database.

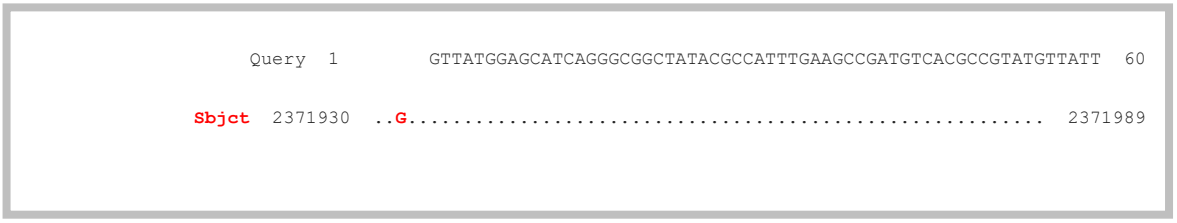

Figure 6. Sequence analysis of *acrA* gene of *E.coli* with Gene Bank of NCBI. Quray represents the sample; the Subject represents a National Center Biotechnology Information (NCBI) database.

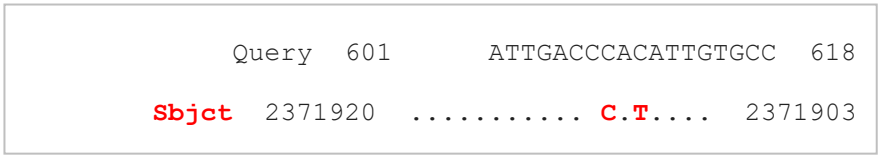

Figure 7: Sequence analysis of *acrB* gene of *E.coli* with Gene Bank of NCBI. Quray represents the sample; the Subject represents a National Center Biotechnology Information (NCBI) database.

Discussion

Urinary tract infections (UTI) in pregnancy are a significant and under-emphasized risk factor for pregnancy morbidity and adverse birth outcomes in low- and middle-income countries (LMIC) settings[13]. In pregnant women, the odds of acquiring urinary tract infections (UTI) from untreated bacteriuria is high, with consequent risk for preterm labour[14]. The Prevalence is higher among individuals in lower socioeconomic classes and those with a history of asymptomatic urinary infection. Increased frequency of screening during pregnancy identifies more cases. Approximately 1-2% of women who do not have bacteriuria at initial screening early in pregnancy will develop bacteriuria later in the pregnancy. Pregnant women are at risk for the increased incidence of UTIs at the beginning of the 6th week, and peaks during weeks 22 to 36 of pregnancy due to several factors, including the uterus sitting directly on top of the bladder and displacing it, shift in the position of the urinary tract and hormonal changes during pregnancy make it easier for bacteria to travel up the urethras to the kidneys.

Additionally, the physiological increase in plasma volume during pregnancy decreases urine concentration. Also, urethral dilatation, bladder volume increased and decreased bladder tone, along with decreased urethral tone which contributes to increased urinary stasis and ureterovesical reflux and up to 70 % of pregnant women develop glycosuria, which encourages bacterial growth in the urine[15,16,17]. Pregnant women diagnosed with bacteriuria are thus offered antibiotics to prevent complications[18]. In our study, Escherichia coli is the most common pathogen associated with asymptomatic bacteriuria (58.9 % of isolates). Other organisms include Klebsiella pneumonia (10%), Proteus mirabilis(6.66%), Staphylococcus saprophyticus (22.22%), and Pseudomonas aurogenosa (2.22%). The DNA of twenty-five isolates of E.coli were extracted, and conventional PCR was performed to amplify the uidA gene and (acrA,acrB) genes. The results showed that (UidA) gene was detected in all 25 uropathogenic Escherichia coli DNA samples by PCR amplification and specific primer. All isolates (100%) were positive for the uidA gene with amplified DNA bands of 658 bp. The results of this study are in agreement with the findings of [19,20]. The uidA gene, which encodes for B-glucuronidase, has been used for detecting E. coli in previous studies[21,.22].This gene encodes an enzyme specific to E. coli. It is, therefore, widely used in identification kits and as a specific marker for E. coli[23].

The *acrA* and *acrB* efflux pump genes that encode the *AcrA* and *AcrB* proteins have been detected respectively within the *AcrAB- ToIC* efflux pump in 25 *E. coli* bac-

terial isolates with technology Polymerase chain reaction (PCR) using a thermocycler to ensure its presence in the all bacterial isolates.

The results of the molecular detection of the *acrA* gene, whose size is (107) base pair, showed all of the bacterial isolates 25(100%) possessed the *acrA* gene by comparing the duplicated packets with the dependent bundles of the DNA ladder, it was found that the resulting packets it had a molecular weight of 107 base pairs.

The results of this study are in agreement with the results reached by the[19,20.24,25] as it reached the percentage of bacterial isolates that possess the acrA gene (100 %,100 % 95.5 %100 %) respectively. While the results of this study disagree with the findings of the[26] in Egypt, as the percentage of bacterial isolates that possess this gene was (74,124), It was shown that there is a close relationship between bacterial possession of the *AcrAB* efflux pump and resistance to different groups of bacteria Antibiotics, as he indicated that this gene is always present with the acrB gene in all bacterial isolates.

The results of the molecular detection of the (*acrB*) gene, whose size is (107bp), showed that 25(100 %) of bacterial isolates possessed the acrB gene by comparing the duplicated packets with the dependent bundles of the DNA ladder, it was found that the resulting packets it had a molecular weight of 107 base pairs. This study's results agree with the results reached by the [19,20,24,25] as it reached the percentage of bacterial isolates that possess the *acrB* gene (100 %, 100 %,82.90 %,100 %), respectively. The results of this study disagree with the findings of the**(26)** in Egypt and Iran. The percentage of bacterial isolates possessing this gene was (74.84)% and (75)%, respectively.

DNA sequencing is considered one of the latest methods to identify disease-causing bacteria. It is also used to detect genetic mutations in resistance and other genes by identifying the sequence of nitrogenous bases specific to the gene[28]. Our study from the Gene Bank found that part of *the UidA* gene and acrA,acrB genes having 99% compatibility with Subject of Uid A gene and acrA,acrB genes in National Center Biotechnology Information(NCBI) under sequence ID: CP086510.1, as seen in Figure (4), Figure (5), Figure (6) and Figure (7).

Conclusion

E. coli has been recognized as a significant public health problem, especially among pregnant women with urinary tract infections (UTIs). Diagnosis of E.coli by conventional methods is considered a proper technique, but these methods are time-consuming and sometimes need to give absolute results for diagnosis compared with the molecular methods. So, polymerase chain reaction using specific primers successfully identifies E.coli(UidA,acrA,acrB) genes.

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