

STUDY OF PREVALENCE SOME PATHOGENICITY-ASSOCIATED VIRULENCE FACTOR GENES IN UROPATHOGENIC *E. COLI* ISOLATES AND ABILITY OF TRANSFERRING THEM TO OTHER RELATED AND NON-RELATED SPECIES IN AL NAJAF PROVINCE-IRAQ

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ABSTRACT

Background: The generally accepted hypothesis today is that UPEC involved from nonpathogenic strains by acquiring new virulence factor from accessory DNA horizontal transfer located at the chromosome or plasmid level. **Aim of study:** investigate the genetic determination of some pathogenicity-associated virulence factors (PAVFs) genes such as *fimH*, *hlyA* and *iucC* genes in UPEC and capability of transferring of them from UPEC to related and non related species such as *E.coli* JM 109 and *P. aeruginosa* as well as evaluation of genes transferring efficiency. **Method:** During the period from May 2014 to November 2014.a total of 290 samples has been collected from patients suffering from Urinary tract infections (170 samples) and burn infections (120

samples) from Alzahraa and Alsadr teaching Hospital as well as private clinics analytical laboratories and Central Health Laboratory in Al-Najaf Al- Ashraf City **Results:** seventy (41.2%) of isolates were belong to Uropathogenic *E.coli* whereas out of 120 burn infection samples only 60(50%) isolates were belong to *P.aeruginosa* depending on identification by microscopic, morphologic, genetic and biochemical tests. So *fimH*, *hlyA* and *iucC* revealed that 48(69%), 6(9%) and 56(80%) isolates were possess these gene respectively, while 12(17%) isolates showed negative results (absence of any target genes) , to confirm ability of these gene to transferred from local UPEC isolates to *E.coli* JM 109 as well as to local cured *P.aeruginosa* that selected from conjugation and transformation possess, results of that showed *fimH* had ability to transfer by conjugation and transformation to *E.coli* JM 109 while

it was unable to transferred to cured *P.aeruginosa* isolated by these two methods. As well as *hlyA* gene had ability to transfer to cured *P.aeruginosa* by transformation process while unable to transfer by conjugation as well as it had no ability to transfer by both methods to *E.coli* JM 109. results of conjugation and transformation showed inability of *iucC* to transfer by two methods to *E.coli* JM 109 and cured *P.aeruginosa*, so transformation efficiency was 55.75 times greater than conjugation efficiency and conjugation efficiency between UPEC strain and *P.aeruginosa* with regard to *hlyA* gene transferability was 1.75 times greater than the transformation efficiency. **Conclusion:** Genetic transformation efficiency between UPEC strain and *E.coli* JM109 with regard to *fimH* gene transferability is greater than the conjugation efficiency, whilst conjugation efficiency between UPEC strain and *P.aeruginosa* with regard to *hlyA* gene transferability was greater than the transformation efficiency.

KEYWORDS: PAVFs, virulence factor, UPEC, *fimH*, *hlyA* and *iucC* genes, horizontal gene transferring.

INTRODUCTION

UPEC strains possess several virulence factors, that mediate it's colonization to the urinary tract and stay face of an immune defenses. Genes that express these characteristics are normally clustered in DNA domains denominated pathogenic islands (PAIs). UPEC samples reflect wide range of genetic variations as a results of having a special virulence genes put on movable genetic elements. These are called pathogenicity islands.^[1]

A pathogenicity island could be a genomic island bearing genes coding for VFs, and there by contributes to the virulence of the host. They are founded in an exceedingly wide range of each bacteria either (Gram +ve and -ve).^[2]

FimH adhesive structure with completely variant strains show variation within the affinity of binding for outlined motifs of oligomannose and these variations within the binding capability might be explained because of the distortions in the fimH variants of primary structure.^[3]

The α -hemolysin desoxyribonucleic acid (operon) of UPEC are often placed at either a chromosome/or plasmid of it. The synthesis, stimulation and secretion of the α - hemolysin are dictated by the *hlyCABD* operon. ExPEC and UPEC particularly express various iron acquisition systems, such as salmochelin and yersiniabactin (which are siderophores too).

Another type of siderophore is aerobactin that is a very important iron uptake system of pathogenic and non-pathogenic *E. coli* and considerably contributes to the virulence of ExPEC.

METHOD

Isolation and Identification of Isolates

Specimens

A-Urine specimens were investigated primary with general urine examination and cultured on MacConkey agar and blood agar, incubated overnight at 37°C aerobically for 48 hrs.^[4]

B- Burn Swabs

burn swab were cultured on MacConkey's and blood agar then incubated for overnight at 37°C.

Biochemical Tests

The preparation of the following tests below were done according to McFadden, (2000).^[5] Which included.

A-IMVIC tests

(Indole Production Test; Methyl Red Test; Voges-Proskauer Test; Simmons Citrate Test; Urease Test; Triple Sugar Iron Test (TSI).

B-API 20 E Identification System

The API 20 E tests are conducted by BioMerieux Company in France.

C-VITEK System

The system was supplied with long identification database for all routine identification examinations that give a developed efficiency in microbial diagnosis.

Polymerase Chain Reaction Technique

Conventional PCR mechanism with Biometra (Germany) equipments is adopted for examining the *fimH*, *hlyA* and *iucC* genes existence that occurred on PAIs encode by (PAI gene) in certain *E.coli* isolates under investigation, and *16SrDNA* gene in *P.aeruginosa* isolates, the process of PCR technique involved the following:

A-Extraction of Bacterial DNA

Boiling method described by Sambrook and Russell (2001)^[6], was used for isolating template DNA from UPEC and *P.aeruginosa*.

B-PCR Assembling

UPEC and *p. aeruginosa* templates DNA were subjected to PCR using 1 sets (F and R) of primers targeting one groups of gene: the first group listed to diagnosis of UPEC and *p. aeruginosa* and the second group listed to determine the virulence properties of UPEC.

C-Agarose Gel Electrophoresis

The electrophoresis were carried out at 60 volts for 120 minutes. The gel was visualized using UV transilluminator units and photographed by Nikon digital camera.

D-Gene Transferring Experiments

Surajit (2015)^[7] method was adopted to study the UPEC capability of transferring their gene (s) of PAVF between local isolates under study to isolates of standard *E.coli* JM 109 and local plasmid cured *P.aeruginosa* by bacterial conjugation and transformation methods (preparing of competent Bacteria by utilizing CaCl₂ was adopted by Sambrook and Russell (2001)^[8] methodology).

Statistical Analysis

In analyzing data we used SPSS computer programme which is called Version twenty-two. SPSS Inc (2015), United States.

RESULTS AND DISCUSSION

Isolation and Identification of UPEC and *P. aeruginosa*

The results reveal totally, out of 170 urine samples only 70(57%) consecutive non duplicated bacterial isolates were with positive UPEC bacteria confirmed by biochemical and Api 20 *E* system tests. The results also indicate that out of (120) burn samples only 60(50%) isolates were positive *P. aeruginosa* bacteria confirmed by *16S rDNA* gene and vitek 2 system test (see figure 1).

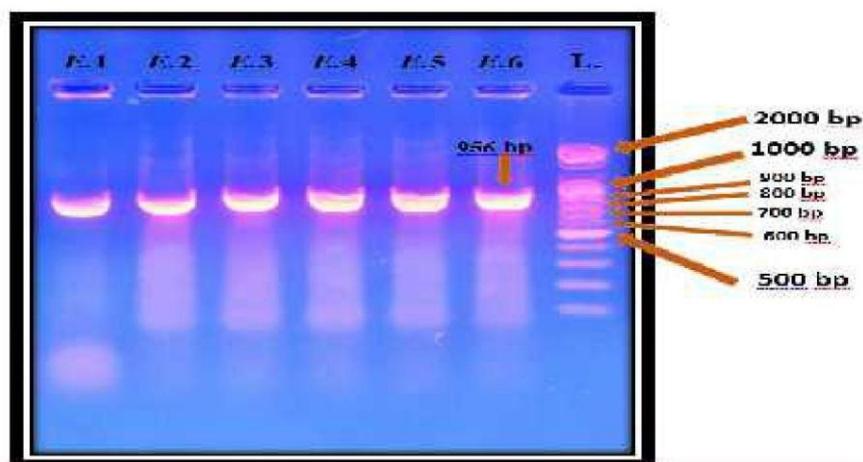


Figure (1): Agarose gel staining with ethidium bromide of PCR exaggerated outcomes from DNA of *P.aeruginosa* isolates and exaggerated with *16 rDNA* primers. The electrophoresis done at 60 volts for 2 hrs. Lane (L), DNA molecular marker of the size (100-2000 bp ladders); Lanes (E1-E6), showed positive results with *16 rDNA* gene at 956 bp of PCR product.

Many researches revealed that UPEC remaining the predominantly isolated species. It was determined that UPEC influence (150) million people every year and leads to 70–90% of community-acquired UTIs and (40) percentage of nosocomial UTIs. In other words, those infections that are acquired in hospitals.^[9] As well as many other studies such as (Gupta and Joshi, 2004; Oncul, et al. 2009 and Khan, 2012) pointed a higher frequency rate of *P. aeruginosa* in patients with burn infections especially in many developing countries such as India, Turkey and Pakistan have documented the following frequency of *P. aeruginosa* which were (59%), (57%) and (54.4%) respectively.^[10]

Genotypic Detection of Some VFs

Table (1) and figure (2, 3 and 4) improved that out of 70 UPEC isolates, only 48 (69%) isolates showed positive results for amplification of *fimH* gene. And only 6(9%) isolates possess *hlyA* gene. and only 56(80%) isolates contained *iucC* gene.

Table (1): Genotypic detection of some virulence factors in uropathogenic *Escherichia coli*.

	No.(%) of isolates that possess			
	<i>fimH</i>	<i>hlyA</i>	<i>iucC</i>	negative
UPEC (n=70)	48(69%)	6(9%)	56(80%)	12(17%)

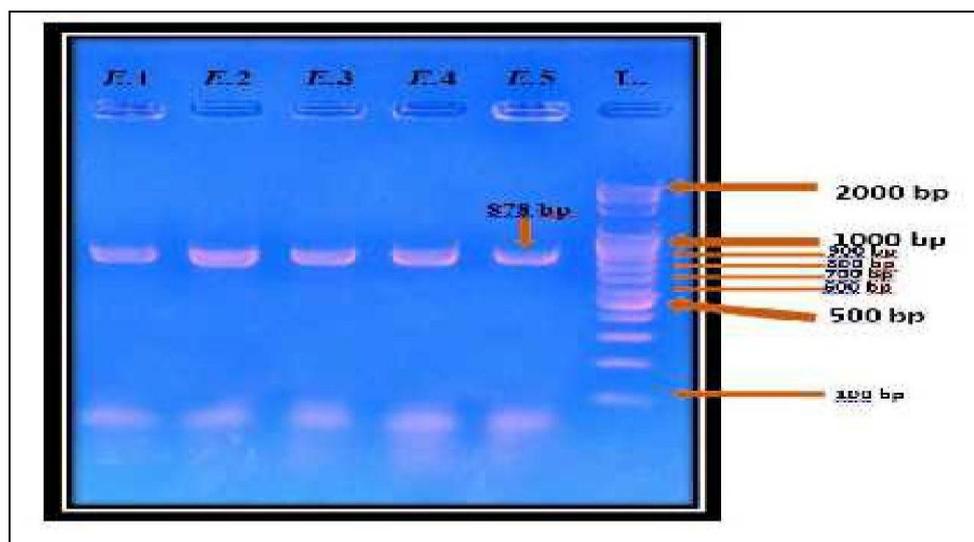


Figure (2): Agarose gel staining with ethidium bromide of PCR exaggerated outcomes from extracted DNA of *E. coli* isolates and exaggerated with *fimH* primers. The electrophoresis done at 60 volts for 2 hrs. Lane (L), DNA molecular size marker (100-2000 bp ladders); Lanes (E1-E5), showed positive results with *fimH* gene at 878 bp of PCR product.

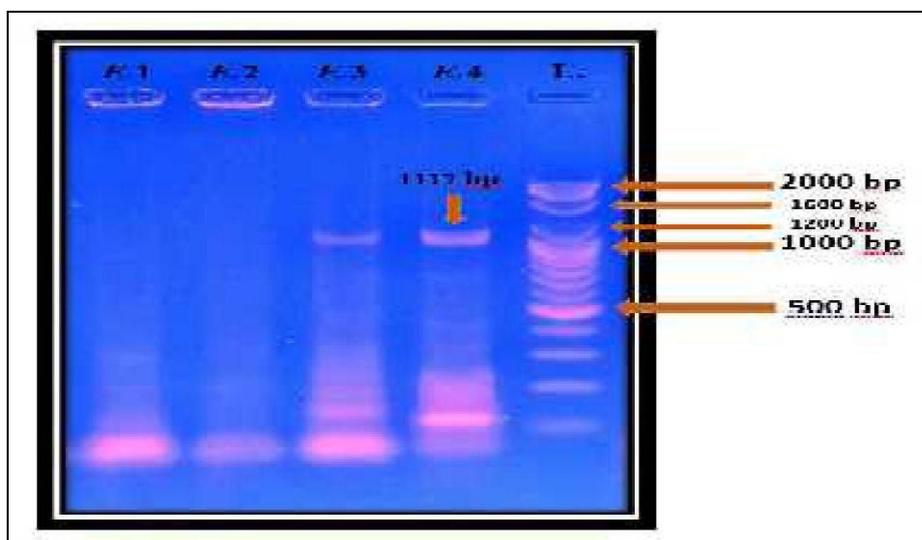


Figure (3): Agarose gel staining with ethidium bromide of PCR exaggerated outcomes from extracted DNA of *E. coli* isolates and exaggerated with *hlyA* primers. The electrophoresis done at 60 volts for 2 hrs. Lane (L), DNA molecular size marker (100-2000 bp ladders); Lanes (E3, E4), showed positive results with *hlyA* gene at 1117 bp; Lanes (E1, E2), show negative results with *hlyA* gene of PCR product.

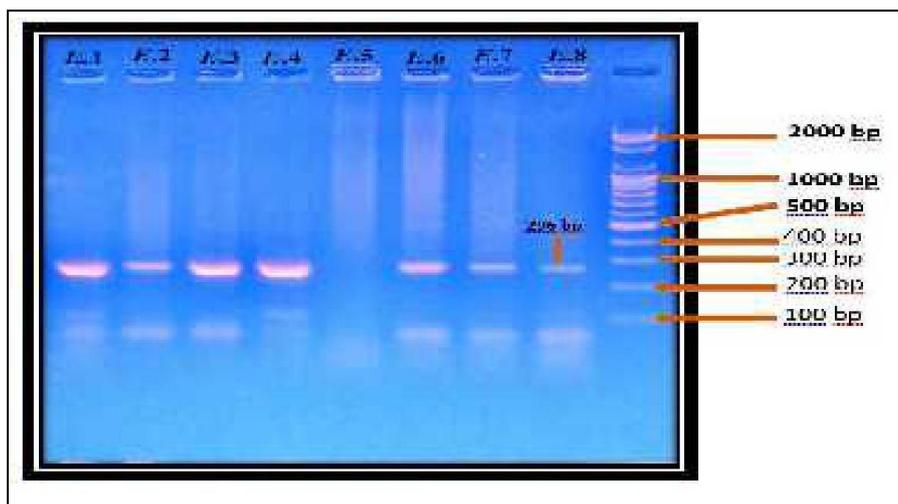


Figure (4): Agarose gel staining with ethidium bromide of PCR exaggerated outcomes from extracted DNA of *E. coli* isolates and exaggerated with *iucC* primers. The electrophoresis done at 60 volts for 2 hrs. Lane (L), DNA molecular size marker (100-2000 bp ladders); Lanes (E1-E4, E6, E7, E8), showed positive results with *iucC* gene at 296 bp; Lane (E5), show negative results with *iucC* gene of PCR product.

Many studies argued that to detect VFs, it is more appropriate to rely upon genotypic assay as being more sensitive and reliable than the phenotypic test.^[11,12] This study focused on the PAVF genes associated with adhesion, hemolysin and siderophore production.

Several studies emphasized the presence of *fimH* gene such as Raksha, et al. (2003) who stated that a virulence genetic determinants like kind 1 fimbriae have more frequency in UPEC^[13], so Donnenberg and Welch, (2004) argue that this gene is one of the high pioneering virulence genes in strains of the UPEC which are correlated with UTIs (severe form)^[14], the *iucC* gene is one of high pioneering gene in virulence of strains within UPEC. It is related to severe UTI and it plays the major role in synthesizing the aerobactin.^[15]

Genes Transfer

E. coli strains achieve new genetic data by HGT, usually in the shape of GIs/plasmids. The GIs group is called `PAIs` as it tends to harbor virulence-associated genes when recognized. The loss of genetic information cannot happen due to absence of GIs/plasmids only, while due to lesions in small genetic region, such as point single mutations.^[16] Lloyd, et al. (2009) argued that PAIs often have proteins contributing to pathogenesis. Any loss of these areas may lead to loss of virulence.^[17]

Conjugation and Transformation

Four UPEC isolates have been selected as a donor cells for transferring a PAIVF gene by conjugation and transformation to *E.coli* JM 109. These isolates were characterized as a) have genotypic and phenotypic properties, b) has genotypic but without phenotypic morphologies, c) has phenotypic but without genotypic properties and d) has no genotypic and nophenotypic properties.

The result of gene transferring of criteria from UPEC isolates that have both phenotypic and genotypic criteria which proved that *fimH* gene was capable of transferring from local clinical UPEC isolate to *E.coli* JM 109 isolate by conjugation and transformation process by appearing of amplicon at 878 bps in DNA extracted from transconjugant and transformant cell while it was unable of transferring from local clinical UPEC isolates to the local cured *P.aeruginosa* isolate by both conjugation and transformation process as shown in figure (5), also *hlyA* gene was unable to transfer from local clinical isolate of UPEC to *E.coli* JM 109 isolate by conjugation and transformation process. Whereas, it was unable of transferring from local clinical isolates of UPEC to local cured *P.aeruginosa* isolate by conjugation method only, but it is transferred by transformation which amplicon with 1117 bps appeared in amplified DNA extracted from transformant cell (figure (6)), also result showed that *iucC* gene was unable to transfer from local clinical isolates of UPEC to standard *E.coli* JM 109 isolate as well as to a local cured *P.aeruginosa* by conjugation and transformation methods. Also, all transconjugant and transformant isolates resulted from conjugation and transformation between UPEC and *E.coli* JM 109 and between UPEC and local cured *P.aeruginosa* isolates were examined phenotypically to evaluate the transferring of phenotypic characters such as α - hemolysis and kind one fimbria production. The results showed appositve transferring of both properties i.e. the transformant and transconjugant cells have the ability to produce hemolysin and kind one fimbria. All genes of UPEC isolates that appeared transferring ability have genotypic and phenotypic properties while other genes properties such as genotypic but without phenotypic properties, phenotypic but without genotypic properties and no genotypic and nophenotypic properties did not appear results in transferring. To confirm efficiency of conjugation and transformation. The frequency has been calculated. Table (3) showed the frequency conjugation which occur between UPEC and *E. coli* JM109. It is evaluated that the rate of frequency represents the percentage of trans conjugants to recipients. These were $(5.56 \times 10^{-6}$ and 0.60×10^{-6}) for *fimH* and *hlyA* respectively. While the rate of frequency in transformant cells resulted from transformation

of *fimH* and *hlyA* from UPEC and *E. coli* JM109 were (3.10×10^{-4}) and (1.87×10^{-4}) respectively (see table (4)). These findings are resulted according to the mating temperature (37°C) and to the proportion of donor and recipient cells (1:3).

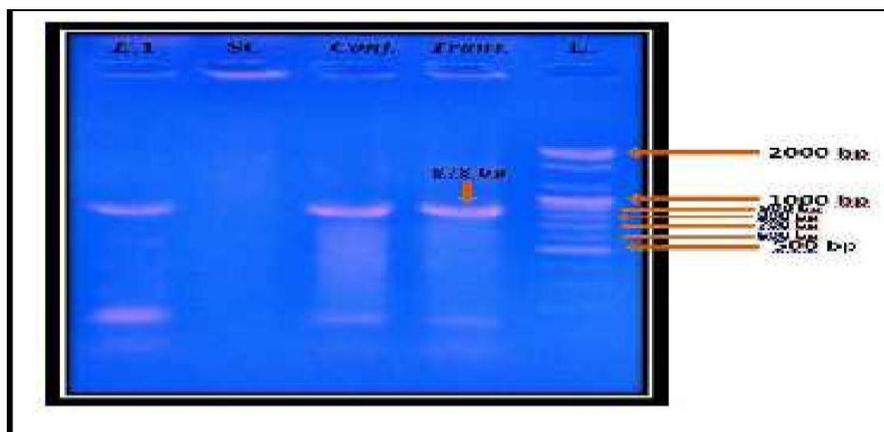


Figure (5): Agarose gel staining with ethidium bromide of PCR exaggerated outcomes from extracted DNA and exaggerated with *fimH* primers. The electrophoresis done at 60 volts for 2 hrs. Lane (L), DNA molecular size marker (100-2000 bp ladders); Lanes [(E1) (*E. coli* isolate), (conj.) (transconjugant isolate) and (trans.) (transformant isolate)] showed positive results with *fimH* gene at 878 bp; Lane (St.) (standard strain *E. coli*) show negative results with *fimH* gene of PCR product.

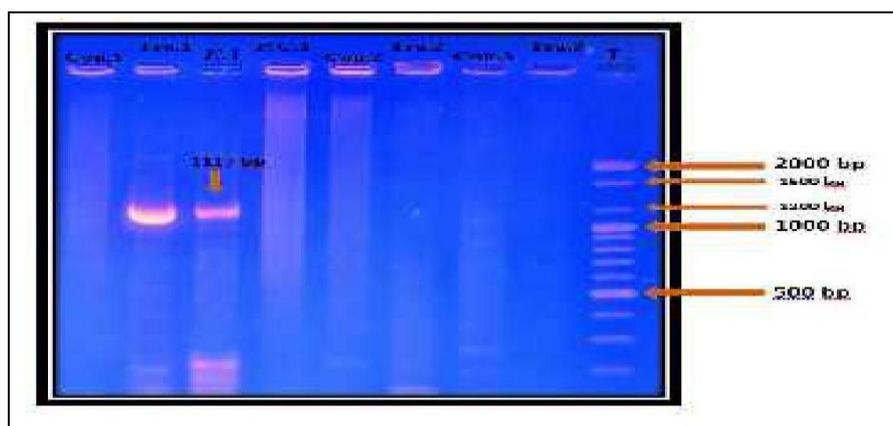


Figure (6): Agarose gel staining with ethidium bromide of PCR exaggerated outcomes from extracted DNA and exaggerated with *hlyA* primers. The electrophoresis done at 60 volts for 2 hrs. Lane (L), DNA molecular size marker (100-2000 bp ladders); Lanes [(E1) (*E. coli* isolate), (trans.1) (transformant 1 isolate of *p. aeruginosa*)] showed positive results with *hlyA* gene at 1117 bp; Lanes [(p.C.1) (SDS plasmid cured isolates of *p. aeruginosa*), (conj.1, conj.2, conj.3) (transconjugant isolates of *p. aeruginosa*) and (trans.2, trans.3) (transformant isolates of *p. aeruginosa*)] show negative results with *hlyA* gene of PCR product.

Table (3): Efficiency conjugation between of uropathogenic *Escherichia coli* and *E. coli* JM109.

Type of donor carrier UPEC isolate	Total No. in 1 mL		Efficiency of Conjugation
	Transconju-gant	Recipient	
<i>fimH</i> gene	12*10 ²	216*10 ⁶	5.56*10 ⁻⁶
<i>hlyA</i> gene	130*10 ²	216*10 ⁶	0.60*10 ⁻⁶

Table (4): Efficiency of transformation between of uropathogenic *Escherichia coli* and *E. coli* JM109.

Type of donor carrier UPEC isolate	Total No. of		Efficiency of Transforma-tion
	Transformant	Recipient	
<i>fimH</i> gene	67*10 ³	216*10 ⁶	3.10*10 ⁻⁴
<i>hlyA</i> gene	405*10 ²	216*10 ⁶	1.87*10 ⁻⁴

On the other hand, the rate of frequency among transconjugant cells resulted from conjugation between UPEC and local cured *P.aeruginosa* isolates was mention in table (5). The results showed that the rate of frequency of transconjugant cells were 19*10⁻³ and 3.37*10⁻³ for *fimH*, *hlyA* genes respectively. While, the rate of frequency of transformant cells were 3*10⁻³ and 1.62*10⁻³ for *fimH*, *hlyA* genes respectively as shown in table (6), depending on the mating temperature (37°C) as well as on the ratio of donor and recipient cells (1:3).

Table (5): Efficiency of conjugation between uropathogenic *Escherichia coli* and local cured *pseudomonas aeruginosa*.

Type of donor carrier UPEC isolate	Total No. of		Efficiency of conjugation
	Transconj-ugant	Recipient	
<i>fimH</i> gene	31*10 ³	16*10 ⁵	19*10 ⁻³
<i>hlyA</i> gene	54*10 ²	16*10 ⁵	3.37*10 ⁻³

Table (6): Efficiency of transformation between uropathogenic *Escherichia coli* and local cured *pseudomonas aeruginosa*.

Type of donor carrier UPEC isolate	Total No. of		Efficiency of transformation
	Transfor-mant	Recipient	
<i>fimH</i> gene	49*10 ²	16*10 ⁵	3*10 ⁻³
<i>hlyA</i> gene	26*10 ²	16*10 ⁵	1.62*10 ⁻³

In spite of the well-investigated nature of UPEC, particularly with regard to VFs and colonization strategies, no any feature perfectly identifies an isolate as UPEC. Instead, UPEC involves a mosaic genome which is the outcome of genomic acquisition, loss and rearrangement.^[18]

With regard to genes transfer, the current study has come to the following findings: First, it is important to point that the term 'pathogenicity island' refers to unstable genomic regions encoding α -hemolysin. kind one –fimbriae and iron uptake systems are present in UPEC strains but they are absent from nonpathogenic isolates.^[19] PAIVF genes transferring under investigation was mainly related to the frequent location of virulence related genes (VAGs) on plasmids, PAIs, or phages. This is why, VAGs is allowed to be cable of varying among strains with the help of HGT.^[20] In this respect, Hacker, et al. (1983) found that the stability of PAIs differs in UPEC strains. The stability or instability occur because of the presence of direct repeats near the junctions of the PAIs. On the other hand, long direct repeats, such as those happening in certain *E.coli* strains, are related to instability and spontaneous deletion of the PAI. Furthermore, it is suggested that deletion mutants are isolated at high frequencies (1×10^{-3}).^[21]

The mistake in the process of transferring unrelated species gene by conjugation or transduction may due to these ways of transferring appear to contain some surface recognition. Furthermore, the gene transferred might not be maintained or expressed because there might be many conducted transcriptional factors contained such as, promotor sequences, ribosome binding sites and codon usage which may be conflicting in particular with different (G + C) content .It should be noted that the absence of gene under investigation in this study results from the low likelihood of cells that have different bacterial strains. This case is considered as the larger obstacle to transfer genes in vitro for meeting each other by naked DNA.^[22]

This study maintains that one spatial non related species of *P.aeruginosa* community together with free DNA diffusion of UPEC strains will cause gene transmission . Particularly, this study noticed that the target gene effect is lessen for intermediate bacterial (transformant), with different efficiency, While in a study conducted by Szollosi, et al. (2006), the HGT done by transformation might be useful for bacteria so as to obtain genes from other spatially far strains to re adapt faster into another situations.^[23]

CONCLUSION

Genetic transformation efficiency between UPEC strain and *E.coli* JM109 with regard to *fimH* gene transferability is greater than the conjugation efficiency. On the other hand, it is found that the conjugation efficiency between UPEC strain and *P.aeruginosa* with regard to *hlyA* gene transferability was greater than the transformation efficiency.

RECOMMENDATIONS

Students should pay attention to submit more molecular details of these events such as DNA sequencing techniques develop correct mechanism to prevent UTIs and the other urological problems which accompany with this disease. It is also recommended that other compulsory surveillance needed to include urinary tract infections not only at clinical hospitals but in the community to get a better understanding of plasmids mediated PAVF in *E coli*.

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