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Study Genotyping of cnf, hly, aer, pap genes in Uropathogenic Escherichia coli from Chronic kidney patients

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ABSTRACT— In humans, urinary tract infections (UTIs) are still the second most prevalent kind of bacterial infection. They afflict persons of all ages and usually necessitate immediate medical attention. Because of the impaired defense mechanism, Asymptomatic urinary tract infections are more prevalent in hemodialysis (HD) patients with chronic renal failure. The study's goal was to isolate and identify *Escherichia coli* from chronic kidney patients, as well as to establish the existence and genotype of virulence genes *(cnf, hly, aer, pap*) in *Escherichia coli* uropathogens. In this study, 150 clinical samples were collected in total chronic kidney patients. UPEC was isolated on MacConkey and EMB agar, then further identified by a microscopic examination and the VITEK-2 System. *Escherichia coli* uropathogens were identified using specific primers. The study found 27 (12.0%) CKD patients infected with bacteria and 123 (54.7%) CKD patients not infected with bacteria. Amplification of *aer* gene found 8 (29.62%) in 27 isolated and *hly* gene was approximated at 2 (7.40%). *CNF* gene in E. coli did not found in all isolates. The prevalence of pap virulence genes was found in 6 (22.22%) of the 27 isolates. The registered sequencing of these genes in NCBI under ID were *hly* gene Lc699243.1, *pap* gene Lc699244.1, *aer* gene Lc699245.1.

KEYWORDS: UPEC, CKD, PCR, *hly, pap, aer, cnf*

1. INTRODUCTION

Escherichia coli is a gram-negative rod-shaped bacterium, facultatively anaerobic bacterium that is highly mobile. It is frequently divided into Enterobacteriaceae, which are known to inhabit both animal and human gastrointestinal tracts, but few E. coli strains have adapted widely to causing diarrhea. and a variety of extraintestinal diseases [7] *Escherichia coli* that can cause disease outside of the gastrointestinal tract are classified as uropathogenic E. coli (UPEC). To overcome the mucosal barrier's sluggishness, UPEC expresses a number of virulence factors [11]. These bacteria's ability to bind to the host's epithelial cells, primarily through the expression of fimbriae, is required for the establishment of infectious diseases [16]. The most common cause of community-acquired urinary tract infections is UPEC (approx. 80-90 percent). On the basis of the presence of genomic pathogenicity islands (PAI) and adhesins, poisons, surface polysaccharides, flagella, and iron absorption mechanisms are all examples of virulence components expressed. four major UPEC phlogroups (A, B1, B2, and D) have been identified [2]. Most of these virulence factors are usually required for UPEC to develop a UTI. UTIs can also be caused by Klebsiella pneumoniae (about 7%), Proteus mirabilis (around 5%), Pseudomonas aeruginosa, Enterococcus faecalis,

Enterobacter, and other bacteria, in addition to UPEC [14]. Non-pathogenic commensal E. coli strains can lead to infection in weaker or immunocompromised hosts, or when gastrointestinal barriers are broken (Bhat *et al*., 2019). Pathogenic Escherichia coli are divided into two groups: enteric/diarrheogenic E. coli and extraintestinal E. coli (ExPEC). Pathotypes of enteric/diarrheic E.coli include enteropathogenic E.coli (EPEC), enterohemorrhagic E.coli (EHEC), enterotoxigenic E.coli (ETEC), enteroaggregative E.coli (EAEC), entero-coli invasive (EIEC), and diffusing adherent E.coli (DAEC). Coli (UPEC), the most popular executive [1]. Based on the site of infection, urinary tract infections are classified as cystitis, pyelonephritis, and bacteriuria. Pathogenic bacterial infection requires host cell adhesion, tissue penetration, and, in some situations, cell invasion, which is followed by cellular growth, transfer to neighboring tissues, and persistence (Wagenlehner *et al*., 2020).

Several virulence factors are encoded by *E. coli* strains, that change a microorganism's ability to invade the tract and change the face of extraordinarily efficient host resistance. UPEC isolates have a great level of genetic variation which results in the presence of specialized pathogenicity placed on movable genomic components known as "pathogenicity islands." Coli virulence factors considered as possibly relevant in the formation of UTIs are divided into two groups: (a) virulence factors affixed to the surface of the bacterial cell (b) release and export of virulence factors to the site of action [17]. In urinary tract infections, The UPEC alpha hemolysin *HlyA* is cytotoxic to numerous cells causing severe tissue injury. This *hlyA* gene is part of an operon that includes the *hlyC, hlyA, hlyB,* and *hlyD* genes. *HlyC* is an acyltransferase that activates *HlyA*, while *HlyB* and *HlyD* are involved in HlyA secretion. (Ristow and Welch, 2016). *HlyA* has been shown to cause inflammation and kidney damage, and a greater proportion of hlyA positive bacteria are isolated in pyelonephritis patients (> 70%) than in cystitis patients (31-48%), showing that *HlyA* has a role in pyelonephritis pathogenicity [9]. The bacterial virulence factor (CNF1) is detected in some pathogenic e. coli which cause urinary diseases and meningitis. Aerobactin is another siderophore found in ExPECs. Like salmochelin, this siderophore was carried by the ColV and ColBM plasmids, and also its synthesis is mediated from enzymes expressed by the iucABCD plasmid (iron uptake chelate) genes. Aerobactin, a hydroxamate siderophore, is produced by the majority of avian pathogenic E. coli (APEC) strains and other pathogenic E. coli. Regardless of chemical differences, so every system is made up of parts that mediate the special steps required for ferric iron uptake, such as cytoplasmic siderophore synthesis, secretion, ferri-siderophore receipt at the external surface of the membrane, internalization, then iron discharge inside the cytoplasm [6].

2. Materials and methods

2.1 Study design

The sample wear collected from midstream for identification of *Escherichia coli* by culture. UPEC was isolated on MacConkey and EMB agar, then further identified by a microscopic examination and the VITEK-2 System. To detect virulence genes (cnf,hly,aer,pap) in Escherichia coli Ur pathogens, the polymerase chain reaction was performed.

2.2 Identification of UPEC

This study included the collection of 150 urine samples (150 patients) from chronic kidney patients. All these samples were distributed in Iraq Baghdad, from the medical city-Baghdad hospital during the period from November 2021 to March 2022 for study, isolation and identification of E. coli strains from chronic kidney patients and detection and genotyping analysis of the virulence genes (cnf, hly, aer, pap) in *Escherichia coli* uropathogens from chronic kidney disease patients.

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2.2.1 Reaction Setup and Thermal Cycling Protocol

extracted DNA from the bacterial isolate, primers, and a PCR Master mix (Promega, USA) containing Taq DNA Green polymerase, MgCl2, deoxynucleotides dNTPs, kCl, stabilizing agent, tracking dye, and Tris-HCl were used (pH 9.0). were melted at 4°C, vortexed, and centrifuged for a short time to get the substances to the bottom of the tubes. This PCR mixture was built up in a 25ul total volume.

Table (1.1) Primers for the identification of *E. Coli*

Primer Name	Seq. 5`……. 3`		Annealing Temp. (C)	Product size (bp)	Reference
Pap	F	GACGGCTGTACTGCAGGGTGTGGCG-	60	328	[3]
	R	ATATCCTTTCTGCAGGGATGCAATA-			
Aer	F	AAACCTGGCTTACGCAACTGT	55	268	[3]
	R	ACCCGTCTGCAAATCAGGAT			
Hyl	F	AGATTCTTGGGCATGTATCCT	50	556	[3]
	R	TTGCTTTGCAGACTGTAGTGT			
Cnf	F	TTATATAGTCGTCAAGATGGA	Gradient Tmp.	693	[3]
	R	CACTAAGCTTTACAATATTGA			

3. Results and discussion

Bacterial isolates were identified on two selective media, MacConkey and EMB agar, using microscopic examination and cultural characteristics. Due to lactose fermentation, it produces dark pink colonies on McConkey agar and a green metallic sheen on EMB agar. The VITEK system was then used to identify UPEC isolates. A study in Baghdad included the collection of 150 urine samples from CKD patients at Baghdad Hospital's Dialysis Center. Of these, 27 (12.0%) were infected with bacteria in CKD patients and 123 (54.7%) were not infected with bacteria in 150 patients.

3.1 Cnf,hly,aer,pap genes in UPEC

The results of *aer* gene amplification in 27 isolated cases, were (29.62 percent). The most common *E. coli* virulence gene yielded positive results, and the amplified products had a molecular size of 269 bp, as shown in Figure, after electrophoresis on agarose gel (1.1). percentage of *hly* gene were2 (7.4%) show figure (1.3), percentage of *pap* gene 6 (22.2%) show (1.4), but all isolates of UPEC not contain *CNF* gene, as seen in Figure, after electrophoresis on agarose gel (1.2).

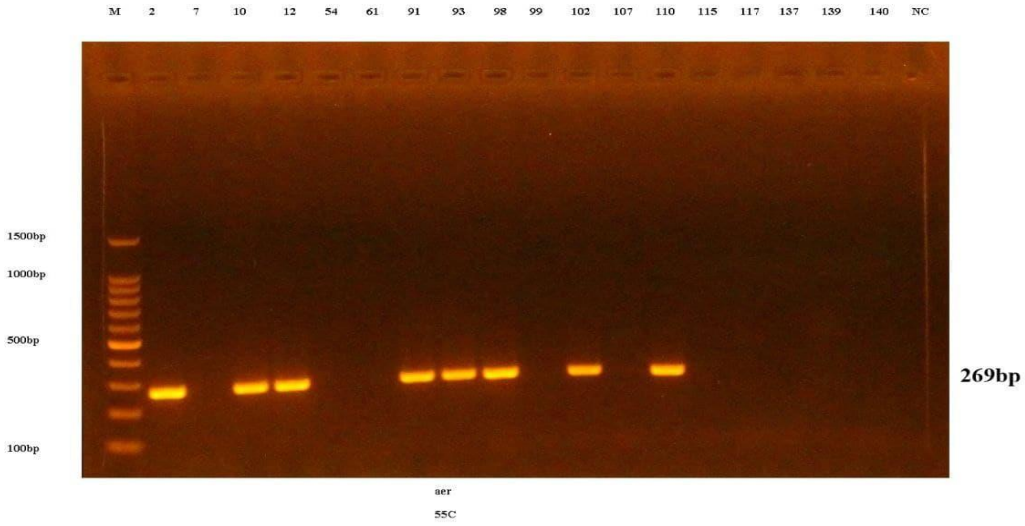

Figure (1.1) Results of aer gene amplification in UPEC samples split on 1.5 percent agarose gel electrophoresis dyed with Eth.Br. M: 100bp ladder marker, NC: negative control

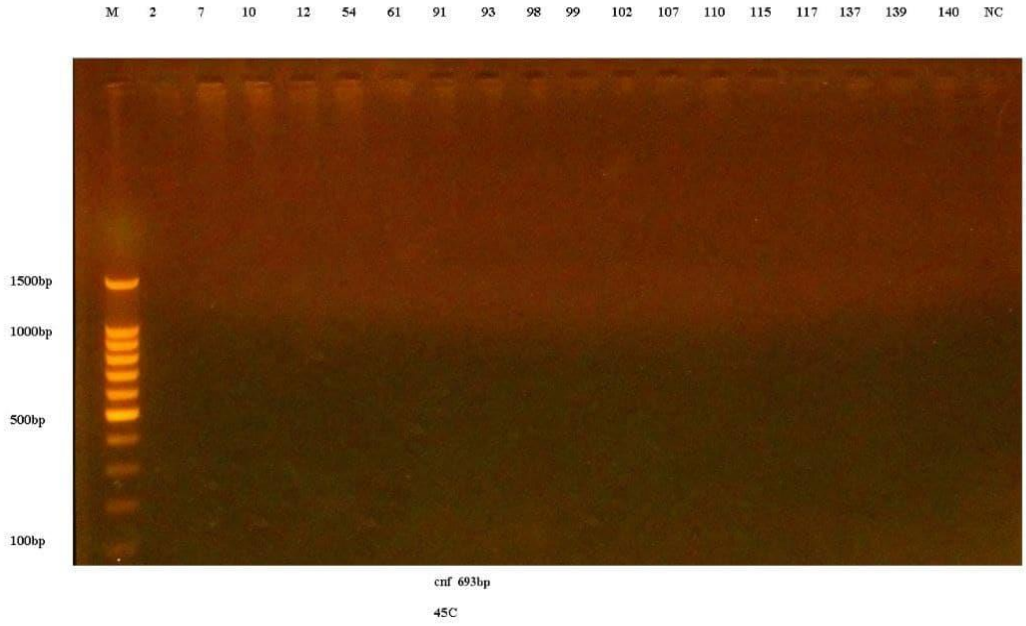

Figure1. 2 The cnf gene was amplified in UPEC samples split on 1.5 percent agarose gel electrophoresis dyed with Eth.Br. M: 100bp ladder marker, NC: negative control

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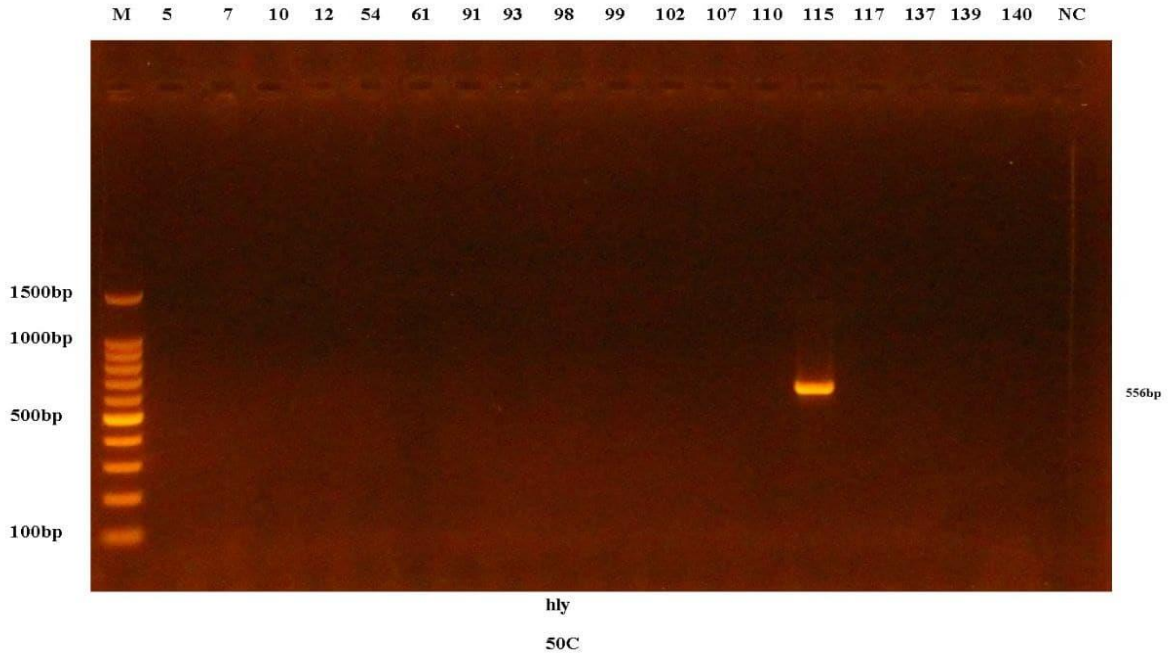


Figure1. 3 The results of the hly gene amplification in UPEC split on 1.5 percent agarose gel electrophoresis dyed with Eth.Br. M: 100bp ladder marker, NC: negative control

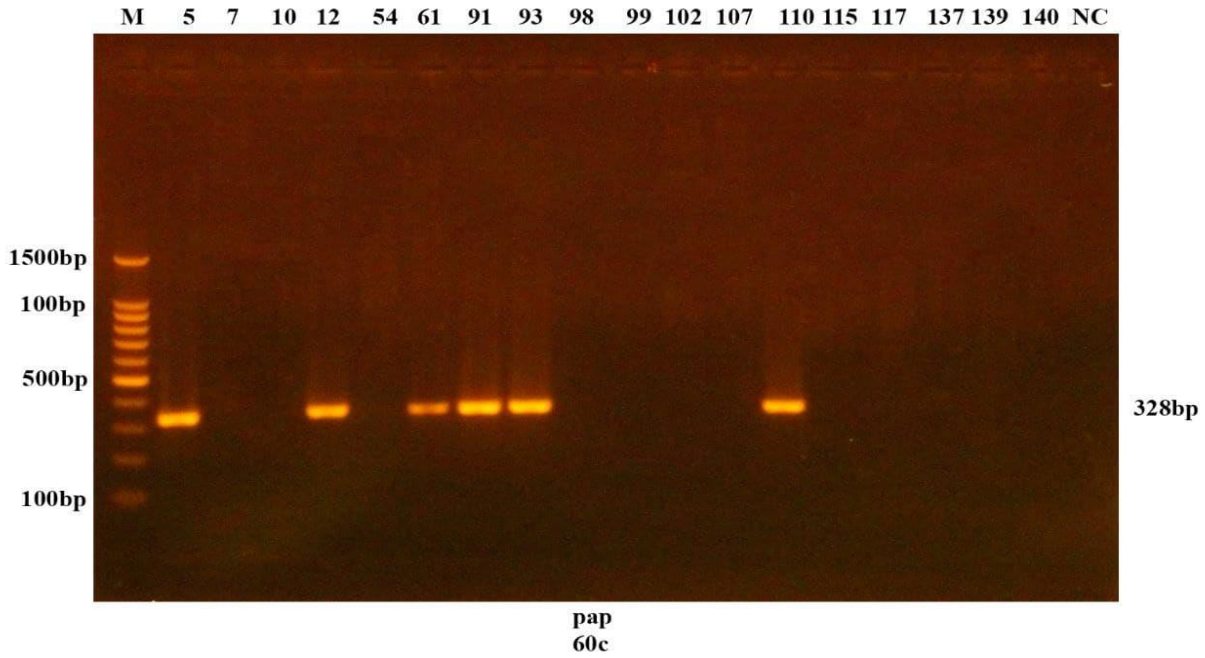
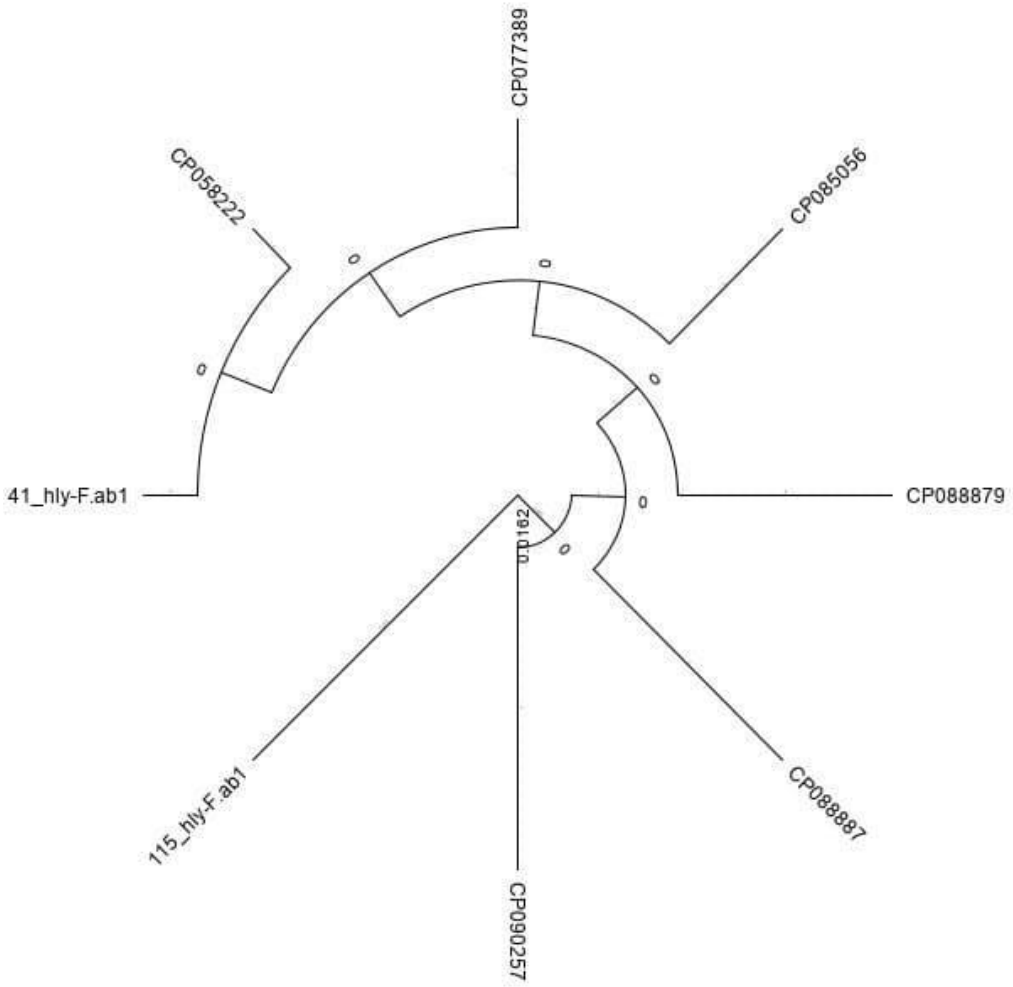


Figure (1.4.) Results of the amplification of pap gene in UPEC on 1.5% agarose gel electrophoresis stained with Eth. Br. M: 100 bp ladder marker, NC: negative control.

3.2 Sequencing of pap, hly, aer, genes.

Sequencing of genes (pap, aer, hly,) were sent for Sanger sequencing to Macrogen Corporation – Korea. Result was received and detected phylogenetic tree in figures (1.5) using geneious software.



Figures 1–5 show a phylogenetic tree of the hly gene in UPEC the ID (CP058222, CP077389, CP085056, CP088879, CP088887, CP090257) refer to UPEC in NCBI.

3.3 Registration locally genes (pap, aer, hly) in NCBI

Documentation genes from Iraqi UPEC details as shown in table (1.2)

Name of isolate	Name of gene	ID in NCBI
Iraq 24	Hly gene	Lc699243.1
Iraq 25	Pap gene	Lc699244.1
Iraq 26	aer gene	Lc699245.1

4. Discussion

E. coli is thought to be the source of 80–90% of UTIs, making it one of the most frequent bacterial illnesses today [15]. Our results on P fimbriae are congruent with those of numerous other studies, which show that roughly 80% and 30% of patients with acute pyelonephritis and cystitis, respectively, have P fimbriae. Moreover, multiple investigations have found that pap adhesion genes have a crucial role in the pathophysiology of E. coli pyelonephritis. Nonetheless, the prevalence of papC in cystitis strains is consistent with previously reported results [4], [19]. Our findings revealed that the *pap* gene was present in

6 people (22.22 percent) Our finding was similar to the data presented by [13], [5] demonstrating that the *pap* gene is considerably more frequent in pyelonephritis patients than in cystitis individuals. Toxins such as alpha hemolysin *(HlyA*) and *CNF1* (cytotoxic necrotizing factor) has been proven to produce direct cytotoxicity in tissues. *HlyA* is frequently carried by UPEC PAIs in conjunction with P-related fimbriae and/or *CNF1*. Prior studies discovered that *HlyA* is usually linked to *Pap, Sfa*, and/or *CNF1*, but not AfaI. [10], [22].

The bacteria loss the *CNF1* may be due to environmental changes (Such as alterations in temperature) or due to mutation. Iron is required for bacterial development but is scarce in the urinary system. As a result, iron acquisition systems play a crucial role in the colonization of ABU E. coli and UPEC. The synthesis of siderophores, low-molecular-weight Fe3+-chelating molecules, and subsequent uptake via their associated membrane receptors is an efficient approach for iron sequestration. [21] in the study this gene the most prevalent in the CKD patient. A mutation is a alter in the nucleotide sequence of a short piece of a genome, and the intensity and location of the mutation may alter the phenotypic outcome. Mutations can happen as a result of errors in replication of DNA or as a result of mutagen exposure (such as chemicals and radiation) [20]. In addition to genes that reduce virulence factor expression, pathoadaptive gene inactivation could address gene expression that indicates a susceptibility during infection (e.g., those detected by immune defense). Any adaptation 'loss-of-function' Protein inhibition can occur by point mutations that alter the active site of the protein, frameshift mutations, or premature stop-codons, resulting in protein truncation as well as deletion of the entire gene or even a block of genes. Gene activities that are damaging to pathogens and are targeted for inactivation are often referred to as "anti-virulence factors.' [18]. The prevalence of the mutator phenotype among UPEC indicates the importance of mutational modifications in urinary tract colonization adaptability. Mutation rates in bacterial clones with the genetic phenotype can increase 10–1000-fold, which is connected with deficits in DNA-mismatch repair genes. Although mutators are fairly unstable in terms of evolution, a change in mutation rate may result in a much higher rate of adaptive mutations and, as a result, give a selection advantage during novel niche colonization [8]. Also, phylogenetic tree exhibited that locally sequences of genes identical in high percentage comparison with global strain in NCBI of same bacteria. There for selected three locally isolates to interpret origin of this genetic variation that may be occur as a result of mutation or inteqrons [12]. Sequence of these genes documented in NCBI included *hly* gene from Iraq 24 strain (LC699243.1), *pap* gene from Iraq 25 strain (LC69924.1), *aer* gene from Iraq 26 strain (LC699245.1).

5. Conclusion

The current investigation found that UPEC isolates have the *pap* and *aer* genes, which can be implicated as major virulence factors in the development of CKD. But the bacteria lost *cnf* gene. The sequence of these genes shown high identify percent with bacteria in NCBI. Then sequence of gene registered in NCBI, *hly* gene ID: LC699243.1, *pap* gene LC699244.1, *aer* gene ID:LC699245.1.

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