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Molecular Study of Some Important Genes among E. coli Isolated from Urinary Tract Infection

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ABSTRACT

A total of 100 samples were collected from patients suffering from urinary tract infections were admitted and visited Maternity and Children Hospital in Al-Hilla city, during a period of three months from September to November 2021. To confirm the identification of E. coli by use selective media, biochemical tests, Api20E system and automated Vitek 2 system, it was found that, 33 isolates were showed positive and identified as E. coli. Molecular detection of some virulence factors genes were studied, out of 33 E. coli isolates, fimH gene was detected in 27(81.8%) isolates by the presence of (508 bp), pap gene was detected in 26(78.8%) isolates by the presence of (336bp), hlyA gene was detected in 10(30.3%) isolates by the presence of (1177bp), feoB gene was detected in 19(57.6%) isolates by the presence of (470bp), usp gene was detected in 21(63.6%%) isolates by the presence of (440bp) and 24(72.7%) have iroN gene by the presence of (665bp) compared with allelic ladder.

Keywords: Genes, PCR, E. coli, Urinary tract infections.

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INTRODUCTION

Urinary tract infection (UTI) represents one of the major nosocomial infections, commonly caused by Escherichia coli, which accounts for 90% of community acquired and 50% of hospital acquired UTI. These E. coli isolates usually originate from patients intestinal normal flora; however, when fecal E. coli colonizes the periurethral area and lead to UTI, these isolates are known as uropathogenic E. coli (UPEC) (Kudinha, 2017; Terlizzi et al., 2017; Ramírez-Castillo, 2018). In addition, to host susceptibility factors, E. coli virulence genes are also an important component in the etiology of recurrent UTIs. The virulence genes most commonly associated with UPEC include P fimbriae (pap), aerobactin (aer), afimbrial adhesin I (afaI), aerobactin (aer), type 1 fimbriae, hemolysin (hly), S fimbriae (sfa), cytotoxic necrotizing factor 1 (cnf1), adhesions and fimbriae (Zhao et al., 2020). In many cases of UTIs, other virulence genes of E. coli including ompT, kpsMT, usp, iroN, iha, astA, group II capsule synthesis; sfa/foc, S and F1C fimbriae; iutA, traT, and serum resistance; fimH are found. These virulence genes are usually associated with occurrence of diseases (AL-Oqaili, 2020). These virulence genes help for bacterial colonization in urinary epithelium and cause severe acuity in UPEC strains which is responsible for causing severe diseases and resistance against antibiotics.

KEYWORDS:
Genes, PCR, E. coli,
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Therefore, detection of these virulence factors is essential especially in cases with UTIs (Makkie & Dehkordi, 2021). Advances in molecular biology, cell biology, and several other areas of science have changed the way we understand the mechanisms in which microbial pathogens interact with their hosts. This trend is set to continue with the advent of microbial genome sequencing, in vivo gene expression analysis, and other related techniques (Ndagi et al., 2020; Levy et al., 2018). However, despite these powerful approaches the basic concept advanced many years ago by Smith that pathogenicity or virulence is a multifactorial property that consists of five basic steps is still valid today. The molecular basis of virulence can still be considered under these five headings: (1) attachment to the host (via mucous membranes); (2) entry into the host (usually); (3) multiplication within the host; (4) Interference with host defence systems; (5) damage to the (Margain Quevedo, 2019). These five stages are not mutually exclusive. The factors produced by pathogens that mediate these steps are termed the determinants of microbial pathogenicity. The molecular basis of these steps will be considered and specific examples will be given to demonstrate the basic principles. Finally it should be emphasised that expression of determinants of pathogenicity is usually regulated and systems exist for environmental sensing and quorum sensing to allow appropriate expression of virulence factors (Argemi et al., 2019; Wilms et al., 2020). The environmental factors and nutrients that determine growth rate in tissues remain largely unknown. Most detailed studies on factors controlling growth in vivo have concerned molecular mechanisms for overcoming iron restriction. Pathogens attempt to obtain iron by one of several mechanisms including (a) from haemoglobin as hemin or heme, (b) directly from ferrated transferrin or lactoferrin, c) indirectly from iron binding proteins by the production of siderophores (d) from intracellular iron stores (Lax et al., 2020). UPEC VFs are grouped by functional categories as adhesions, toxins, iron acquisition systems, and protectins. VFs are encoded by genes located on chromosomes or plasmids, with some being exclusively chromosomal (e.g., pap and hly), others exclusively or principally plasmid-associated, e.g., iss and traT, and some either chromosomal or plasmid- associated (afa). Consequently, VFs may be vertically or horizontally transmitted, further contributing to the complexity of understanding the role played by specific VF genes in UTI pathogenesis (Kudinha, 2017; Botero, 2017).

Aim of study

The aim of this study to detected for some important genes among E. coli isolates from urinary tract infection.

MATERIALS AND METHODS

Study Design:

A total of 100 samples were collected from patients suffering from urinary tract infections were admitted and visit in Maternity and Children Hospital in Al-Hilla city, during a period of three months from September to November 2021.

Ethical Approval

All subjects involved in this work were informed and the agreement required for doing the experiments and publication of this work was obtained from each one prior the collection of samples. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee (at College of Medicine University of Babylon).

Clinical specimens

The specimens were generally collected from patients suffering from UTIs. Mid-stream urine samples were collected in sterilized screw-cap containers, then the urine samples were inoculated on culture media and incubated aerobically at 37oC for 24 hours.

Identification of bacteria
Colonial morphology and microscopic examination

Depending on its morphological properties (colony form, size , color, borders, and texture), a single colony from each primary positive culture on blood, MacConkey and nutrient agar and classify it and examine it by light microscope after being stained with Gram's stain. Biochemical tests and Epi20 system were performed on each isolate after inspection to complete the final identification according to (Baron et al, 1994; McFadden, 2000) and we used the vitek2 method for E. coli identification.

Identification of bacterial isolates with Vitek2 System

Vitek 2 clinical microbiology used as an automatic identification (ID) instrument device.

DNA Extraction

This method was made according to the genomic DNA purification Kit supplemented by the manufacturing company Geneaid, (Korea).

Detection of some of E. coli virulence genes

DNA (extract from bacterial cells) was used as a template in specific PCRs for the detection of some of E. coli virulence genes. DNA was purified from bacterial cells by using the Geneaid DNA extraction Kit. The primers used for the amplification of a fragment gene were listed in Table (1). Rahdar et al., (2015)

Table 1: the primers, sequences, and PCR conditions

Gene name	*Primer sequence (5´- 3´)*	*Size of Bp*	*Conditions*
Pap	F: GCAACAGCAACGCTGGTTGCATCAT R:AGAGAGAGCCACTCTTATACGGACA	336	Step 1: 95ºC, 2 min. Step 2: 95ºC, 30 sec. Step 3: 63.2ºC, 30 sec. Step 4: 72ºC, 40.0 sec. Step 5: Repeat steps 2-4 29 more times Step 6: 72ºC, 5 min. Step 7: 4ºC
fimH	F: TGCAGAACGGATAAGCCGTG R:GCAGTCACCTGCCCTCCGGTA	508	Step 1: 95ºC, 5min. Step 2: 95ºC, 30 sec. Step 3:55ºC, 30 sec.Step 4:72ºC, 40 sec. Step 5:72ºC, 5min
hlyA	F: AACAAGGATAAGCACTGTTCTGGCT R: ACCATATAAGCGGTCATTCCCGTCA	1177	Step 1: 95ºC, 2 min. Step 2: 95ºC, 30 sec. Step 3: 61.6ºC, 30 sec. Step 4: 72ºC, 120.0 sec. Step 5: Repeat steps 2-4 29 more times Step 6: 72ºC, 5 min. Step 7: 4ºC Step 3: 63.3ºC decrease 0.5ºC per cycle, 30 sec.Step 4: 72ºC, 50.0 sec. Step 5: Repeat steps 2-4 14 more times Step 6: 95ºC, 30 sec. Step 7: 56.3ºC, 30 sec. Step 8: 72ºC, 50.0 sec. Step 9: Repeat steps 6-8 19 more times Step 10: 72ºC, 5 min. Step 11: 4ºC
feoB	F: AATTGGCGTGCATGAAGATAACTG R: AGCTGGCGACCTGATAGAACAATG	470	Step 1: 95ºC, 2 min. Step 2: 95ºC, 30 sec. Step 3: 64.5ºC decrease 0.5ºC per cycle, 30 sec.Step 4: 72ºC, 50.0 sec. Step 5: Repeat steps 2-4 14 more times Step 6: 95ºC, 30 sec. Step 7: 57.5ºC, 30 sec. Step 8: 72ºC, 50.0 sec. Step 9: Repeat steps 6-8 19 more times Step 10: 72ºC, 5 min. Step 11: 4ºC
usp	F: ACATTCACGGCAAGCCTCAG R: AGCGAGTTCCTGGTGAAAGC	440	Step 3: 63.3ºC decrease 0.5ºC per cycle, 30 sec.Step 4: 72ºC, 50.0 sec. Step 5: Repeat steps 2-4 14 more times Step 6: 95ºC, 30 sec. Step 7: 56.3ºC, 30 sec. Step 8: 72ºC, 50.0 sec. Step 9: Repeat steps 6-8 19 more times Step 10: 72ºC, 5 min. Step 11: 4ºC
iroN	F: AAGTCAAAGCAGGGGTTGCCCG R: GACGCCGACATTAAGACGCAG	665	Step 1: 95ºC, 2 min. Step 2: 95ºC, 30 sec. Step 3: 66.7ºC decrease 0.5ºC per cycle, 30 sec.Step 4: 72ºC, 70.0 sec. Step 5: Repeat steps 2-4 14 more times Step 6: 95ºC, 30 sec.

Step 7: 59.7ºC, 30 sec.
Step 8: 72ºC, 70.0 sec.
Step 9: Repeat steps 6-8 19 more times
Step 10: 72ºC, 5 min.
Step 11: 4ºC

RESULTS AND DISCUSSION

Isolation of pathogenic bacteria

A total of 100 samples were collected from patients suffering from urinary tract infections were admitted and visit in Maternity and Children Hospital in Al-Hilla city, during a period of three months from September to November 2021. 33 isolates were showed positive and identified as E. coli by using selective media, biochemical test, Epi20 system and Vitek 2 system.

Detection of virulence factors genes
Detection of fimH gene:

Molecular study of fimH genes was detected in 27(81.8%) out of (33) E. coli isolates, the positive results were detected by the presence of (508 bp) bands when compared with allelic ladder as shown in Figure (1).

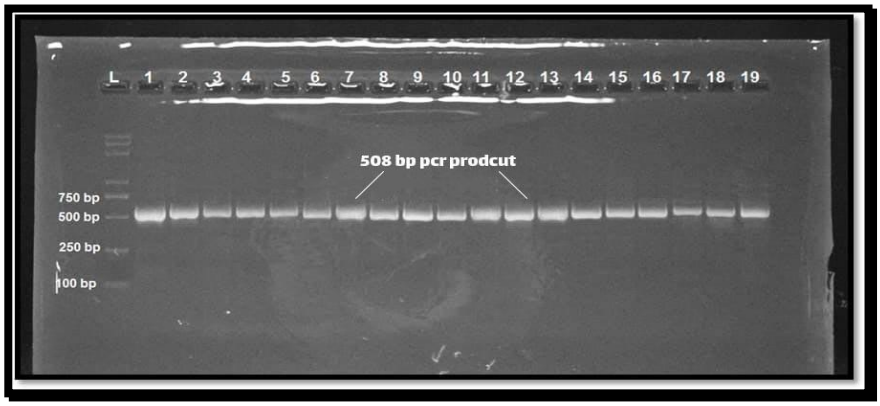

Fig.1: Gel electrophoresis of PCR product of fimH gene at 508bp of E. coli.
L: ladder, line (11,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19) positive results for amplification, the electrophoresis was at 70 volt for 80 min.

The fimH gene was the most prevalent virulence gene and was found in 68% of the UTI isolates (Ahmed et al., 2019). Sameer & Aziz, (2020) studied UPEC isolates collected from females and found that, the fimH gene was the most prevalent virulence factor and 100% of the isolates had that gene. In another study, Düzgün et al. (2019) demonstrated that the fimH gene was the most frequent virulence gene and was detected in 98% of E. coli strains isolated from patient with UTIs. Type 1 fimbriae is present among UPEC as well as other strains of E. coli, to the extent that the fimH gene was detected in more than 90% of the E. coli strains. The prevalence of the fimH gene was examined. The presence of the fimH gene was confirmed by PCR and the results indicated that the fimH gene was present in (92.8%) UPEC isolates (Rasoulinasab et al., 2021), other study of Ghavidel et al., (2020) found that (47.7%) of UPEC isolates were contain fimH gene. The high binding ability of fimH could result in increased bacterial binding to target cells and increased pathogenicity of E. coli; thus, fimH could be used to design vaccine for prevention of E. coli infections by blocking the bacterial attachment and colonization. In addition, fimH could be used as a tool for the extension of rapid detection-based assays (Hojati et al., 2015).

Detection of P fimbriae gene (Pap gene)

Molecular study of P fimbriae gene (pap gene) was done for all 33 isolates that previously identified as E. coli. PCR is a sensitive and specific method for identification of pap gene. The results showed that, out of 33 E. coli isolates, 26(78.8%) were gave positive results for this gene. The positive results were detected by the presence of (336bp) bands when compared with ladder as shown Figure (2). These results were agreement with previous results of Khairy et al., (2019), Tewawong et al., (2020), Kocúreková et al., (2021), who found that, E. coli strains were investigated for the presence of the pap alleles by a multiplex PCR protocol, and the results showed that, there were positive results for this gene. Nascimento et al., (2021) found that, Pap genes were determined by a DNA probe in 85% of the E. coli strains isolated from UTIs. These strains showed diffuse and localized adherence to tissue culture cells without any association to the expression of P-fimbriae on agar medium.

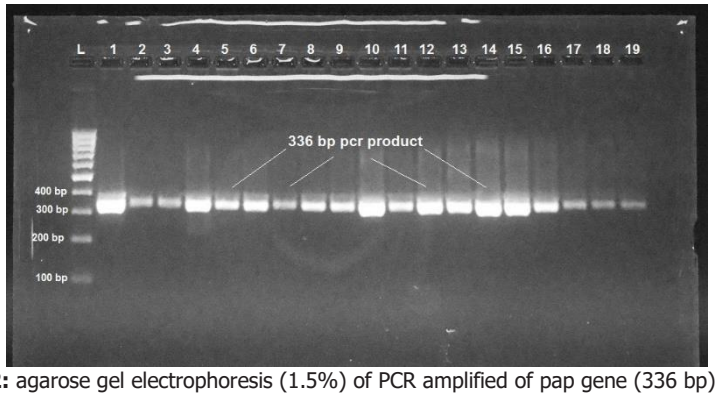

Fig.2: agarose gel electrophoresis (1.5%) of PCR amplified of pap gene (336 bp) of E.coli for(60) minat (100) volt. L:ladder, (1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19) was positive results for pap gene.

Many microorganisms have the genetic capacity to express different adhesions, providing access to multiple receptors and therefore increasing their pathogenicity. The pap operon that encodes the P fimbriae and the pap genes were investigated among human, E. coli strains that produce adhesions (Zamani & Salehzadeh, 2018). The presence of combined pap virulence gene was observed in 100% of isolates, indicating high presence of virulence genes in isolates collected from patients with UTIs. The presence of higher number of observed combined pap gene was in accordance with other reports (Malekzadegan et al., 2018; Shahbazi et al., 2018). The higher number of isolates having pap gene as compared to other combinations could be due to the localization of these gene on the pathogenicity island of UPEC strains (Gati et al, 2019). The distribution of the pap among E. coli strains were produce additional adhesive factors implicated in bacterial binding to intestinal cells suggested a role for P-related fimbriae in the spread of bacteria to extra intestinal sites. In addition, the pap gene was highly associated with E. coli isolates that produce adhesions (Zamani & Salehzadeh, 2018). Therefore, a genotypic and phenotypic characterization of the P-related fimbriae produced by E. coli strain was performed and demonstrated that (i) a complete pap gene cluster necessary for the synthesis of P-related fimbriae seems to be present in the bacterial chromosome, (ii) E. coli contains a variant or a partial copy of the papG adhesion gene, and (iii) Pap is highly related to Pap subunits but the environmental conditions for optimal production are different (Liu et al., 2021).

Detection of hlyA gene

Molecular detection of hemolysin gene (hlyA gene) was done for 33 isolates that previously detected as E. coli. The results showed that out of 33 isolates, only 10(30.3%) were gave positive results for this gene. The positive results were detected by the presence of (1177bp) bands when compared with ladder as shown in Figure (3). These results similar to the results obtained by Yazdanpour et al., (2020) who found that, the ratio of the presence of a hlyA gene in E. coli isolates of urine 26%. Shahbazi et al., (2018) found that, the ratio of the presence of a hlyA gene in E. coli isolates of urine at rate 41.7% and Moeinizadeh & Shaheli, (2021) found that, E. coli isolates of have hlyA gene in rate (90.8%).

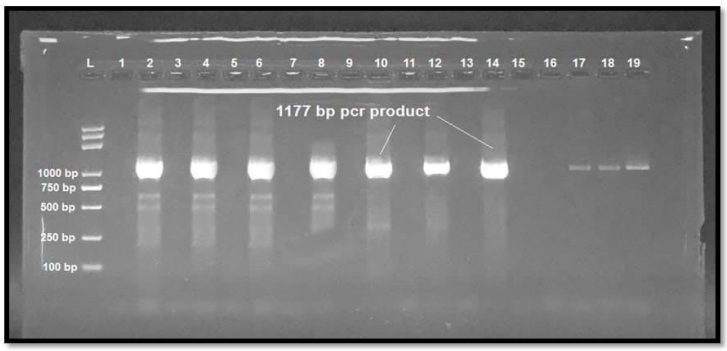

Fig.3: agarose gel electrophoresis (1.5%) of PCR amplified of hlyA gene (1177 bp) of E. coli for (60) min at (100) volt. L: ladder, (2,4,6,8,10,12,14,17,19) was positive results for hlyA gene.

Hemolysin is the most common toxins produced by E. coli, and it was considered virulence factor that isolated from E. coli. It has been suggested that the hemolytic strains of

E. coli was more likely to develop in urinary tract infections, particularly cystitis and acute pyelonephritis (Schwidder et al., 2019). As that the production of hemolysin contributes to the virulence of the bacteria and favours their proliferation in the intestinal. Hemolysin may play a role in intestinal infections of human with intestinal pathogenic E. coli. So, the α-hly has been shown to cause cellular damage and trigger inflammation in the mammalian host (Bücker et al., 2020). Hemolysin-

producing E. coli have been shown to be virulent in human infections and associated with increased severity of infection. Younis et al., (2021) showed that (33.3%) of E. coli human isolates were hemolytic. The lack of hemolysin phenotypic/genotypic congruence may suggest the occurrence of missing genes in the hyl operon among gylA positive/ hemolysin-negative strains. Not all hyl gene carrying isolates in this study expressed beta hemolytic activity. However, not all the isolates that phenotypically expressed the ß-hemolytic activity harbored the hyl gene. A contrasting situation was reported previously where some hemolysin gene carrying isolates did not express ß-hemolytic activity phenotypically.

Detection of feoB gene

Molecular detection of feoB gene was done for isolates that previously detected as E. coli. The results showed that, out of 33 isolates 19(57.6%) gave positive results for this virulence gene. Positive results were detected by the presence of (470bp) bands when compared with allelic ladder as shown Figure (4). These results were similar to results obtained by Sestok et al., (2018) who found that, the Feo system is the only transport pathway that is widely distributed and that is dedicated to the transport of ferrous iron. The Feo system was first discovered in Escherichia coli, and it consists of the feoA, feoB, and feoC (yhgG) genes (Gerken et al., 2020). The role of FeoA is not well understood within the Feo system. E. coli FeoA is a 75-residue cytoplasmic protein with an unknown function. It was proposed that FeoA may interact with FeoB to take part in ferrous iron transport (Sestok et al., 2018).

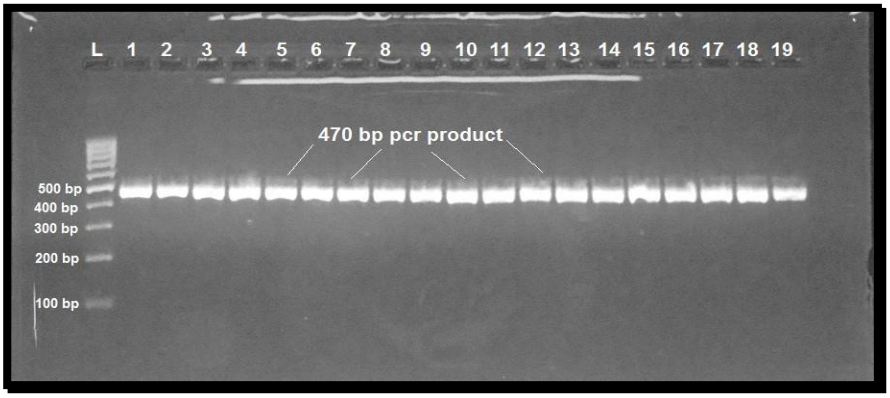

Fig.4: agarose gel electrophoresis (1.5%) of PCR amplified of feoB gene (470bp) of E. coli for (60) min at (100) volt. L: ladder, (1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19) was positive results for feoB gene.

E. coli has an iron (II) transport system (feo) which may make an important contribution to the iron supply of the cell under anaerobic conditions (Gerken et al., 2020). Cloning and sequencing of the iron (II) transport genes revealed an open reading frame (feoA) possibly coding for a small protein with 75 amino acids and a membrane protein with 773 amino acids (feoB) (Fadel, 2018). The upstream region of feoAB contained a binding site for the regulatory protein Fur, which acts with iron (II) as a corepressor in all known iron transport systems of E. coli (Berges et al., 2018). Bacterial ferrous iron transport systems are currently much less understood in comparison with ferric iron transport pathways, it is not yet known whether ferrous iron enters the cell through active transport or through a passive diffusion mechanism. In vivo models have shown that feoA and feoB are closely linked and that deletion of FeoA reduces ferrous iron uptake while deletion of FeoB completely abolishes ferrous iron transport (Sestok et al., 2018). feoA has previously been classified as a Src- homology 3 (SH3)-like domain based on its low amino acid sequence similarity to the C- terminal domain of DtxR, while feoB is thought to provide energy and regulate transport. E. coli feoA contribute to the understanding of bacterial ferrous iron transport as we have been able to investigate both the solution structure and the dynamic properties of the protein and the role that it may play in ferrous iron transport (Smith et al., 2019). feoB is the most conserved Feo protein. It is generally believed that its membrane-embedded C-terminal domain forms the pore for iron transport, although this has not been demonstrated experimentally. Initial in vitro characterization of the N-terminal domain indicated that its low affinity for GTP and the low rate of GTP hydrolysis would prevent it from generating sufficient energy for the iron transport reaction. This led to the model in which the GTPase domain functions as a G protein, regulating rather than energizing iron transport. However, its low affinity for GDP suggested a mechanism distinct from those of the well-characterized eukaryotic G proteins. Further investigation indicated that the linker region between the GTPase motifs and the C-terminal transmembrane domain acts as a nucleotide dissociation inhibitor (GDI)-like domain that may stabilize GDP binding (Sestok et al., 2021).

Detection of usp gene

Molecular studies of virulence usp gene were done for all 33 isolates of E. coli by using specific PCR method is used to detect the virulent gene encoding usp using specific primer PCR technique clearly identified the virulent gene as usp gene of E. coli. The genomic DNA of the sample was extracted and bands were observed by performing agarose gel electrophoresis, when PCR was performed, results clearly indicate that, 21(63.6%%) contained usp gene, the amplified products produced a band at the level of (440bp), when compared with the allelic ladder as showed in Figure (5). These results agreed with the results obtained by Ahmed et al., (2019) who found that, the presence of a usp gene in E. coli isolates of urine in rate 61%. Dong et al., (2019) found that,

the presence of a usp gene in

E. coli isolates of urine at rate 55% and de Cássia Bicudo et al., (2019) found that, E. coli isolates of have usp gene in rate 2.4%. The E. coli uropathogenic-specific protein (Usp) is a bacteriocin-like genotoxin, active against mammalian cells and associated with E. coli strains that provoke pyelonephritis, prostatitis and bacteraemia (Rihtar et al., 2020). The protein designated 'Universal Stress Protein (USP)' is significantly overexpressed under unfavorable environmental stresses, such as nutrients starvation (deficiency of carbon, nitrogen, phosphate, sulfate, and amino acids), heat/cold shock, oxidative stress, heavy metal toxicity, uncoupler of electron transport chains, exposure to polymyxin, cycloserine, ethanol and antibiotics (Ahmed et al., 2019).

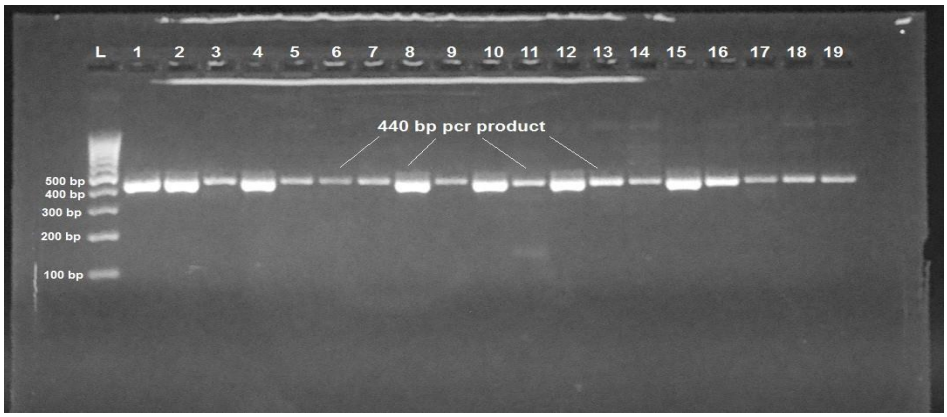

Fig.5: agarose gel electrophoresis (1.5%) of PCR amplified of usp gene (440bp) of E. coli for (60) min at (100) volt. L: ladder, (1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19) was positive results for usp gene.

Usp is encoded by a small pathogenicity island with three downstream small open reading frames (Imu1-3) that are believed to provide immunity to the producer. To prevent host suicide, colicins, bacteriocins of E. coli, form tight complexes with their cognate immunity proteins. Colicin – immunity protein complexes are among the strongest protein complexes known. Here, the Usp associated immunity protein 3 (Imu3) was partially characterized to gain insight into its role and mechanism of activity. All three immunity proteins are thus not essential for the protection of the Usp producers, although Usp is lethal when it is expressed alone in E. coli. None of the three proteins is exclusively required for Usp protein synthesis. As protection of the Usp-producing bacterial cell might be provided by a mechanism that is different from that of the colicins (Espinoza & Minami, 2018). Study by Kudinha, (2017) showed a strong association between Usp and bacteremia of urinary tract origin, suggesting that Usp is important in the migration of UPEC from the urogenital tract to the blood stream. Other studies have shown comparable prevalence of Usp in cystitis, pyelonephritis, and prostatitis isolates. Furthermore, Usp has (frequently) been associated with all common serotypes of UPEC (Ali et al., 2019; Etefia & Ben, 2020).

Detection of iroN gene:

Molecular detection of iroN gene was done for (33) E. coli isolates and the results showed that, 24(72.7%) have this gene. The positive results for (iroN) virulence were detected by the presence of (665 bp) band compared with allelic ladder as shown in Figure (6). The present results agree with Robinson et al., (2018) and Hashimoto et al., (2021). Zou, (2021) found that, iroN, is associated with UPEC. The iron utilization systems sought in this study were much common in UPEC. The iron utilization systems of invasive pathogens such as E. coli that causes extraintestinal infections have been extensively investigated.

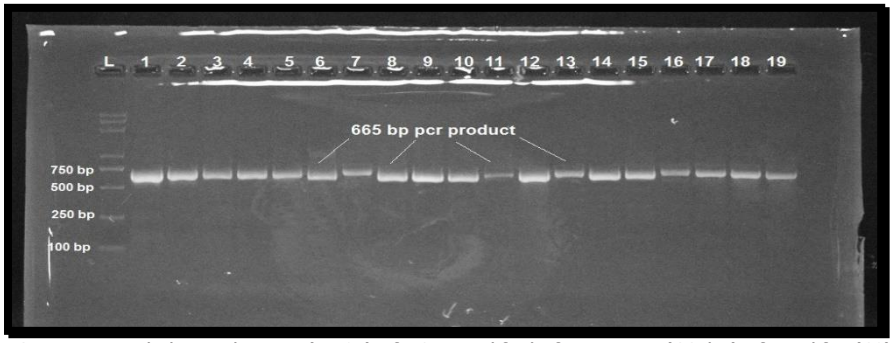

Fig.6: agarose gel electrophoresis (1.5%) of PCR amplified of iroN gene (665bp) of E.colif or(60)min at(100)volt.L:ladder, (1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19) was positive results for iroN gene.

The role of iron as a critical nutrient in pathogenic bacteria is widely regarded as having driven selection for iron acquisition systems among uropathogenic Escherichia coli isolates. Carriage of multiple transition metal acquisition systems in

UPEC suggests that the human urinary tract manipulates metal-ion availability in many ways to resist infection (Robinson et al., 2019). For Siderophore systems in particular, recent studies have identified new roles for Siderophore copper binding as well as production of Siderophore-like inhibitors of iron uptake by other, competing bacterial species. Among these is a process of nutritional passivation of metal ions, in which uropathogens access these vital nutrients while simultaneously protecting themselves from their toxic potential (Kaplan et al., 2021). Organisms generally respond to iron deficiency by increasing their capacity to take up iron and by consuming intracellular iron stores. E coli, in which iron metabolism is particularly well understood, contains at least 7 iron-acquisition systems encoded by 35 iron-repressed genes (Cronin et al., 2019).

CONCLUSION

Urinary tract infections are one of the common infections which are encountered in clinical practice. The present study has also shown the production of various virulence factors genes among isolates related to their pathogenicity.

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