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Revelation of Specific Ferocity Genes in *Escherichia coli* Isolated from Patient with Urinary Tract Infections (UTIs)

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ABSTRACT

Background: The purpose of this research is to detect the occurrence of ferocity genes in uropathogenic *E. coli* that isolated from urinary tract infections by Polymerase chain reaction technique.

Method: A whole of five strains of *E.coli* were obtained from patient with urinary tract infections. Identification of *E.coli* was depended on colonial morphology in culture media, gram stain, and conventional biochemical tests. ferocity factors genes (cnf-1, hly,urea A and ndv B) of *E.coli* strain were revelated by molecular technique.

Results: ferocity genes were detected successfully by molecular technique in five isolates of *E.coli*, the predominance of genes in isolates are seen in percentage 100%

Conclusion: Urinary tract infections still the major universal infection in the worldwide. The one of the most prevalent infectious diseases induce renal failure in patients is UTI that generated by UPEC. The presence of ferocity factors are critical features where the grade of pathogenicity of UPEC strains relied on it. This study indicate that was a real relationship between the ferocity factor genes of *E.coli* and clinical symptoms of UTI.

Keywords- *Escherichia coli*, PCR, urinary tract infection, ferocity genes.

I. INTRODUCTION

Urinary tract infection considered the common bacterial infection in population and also is one of the frequently nosocomial infections **(**Dadi et al.,2020). UTIs comes the second bacterial disease of personal subsequently respiratory tract infection, UTI evolved through bacteria, despite some virus and fungi have in addition been implicated, Gram negative- bacteria *E.coli* in all patients groups is the most dominant causative agent, causing 80-90 % of all UTI. Uropathogenic *E.coli* (UPEC) strains consideration the biggest causative of UTI. Several ferocity genes in *E.coli* causing disease, these ferocity factors are adhesins, capsule, toxins and others (Kudinha,2017). The *E.coli* genome contain a vital mobile gene and a core genome pool that regulate ecotype and pathotype specific traits. Pathogenicity –associated islands (PAIs) may bear in UPEC isolates bear of distinct ferocity cooperative genes, number of ferocity genes are not specific to pathogenic isolates these genes are found in universal isolates (Rezatofighi et al., 2021). ferocity factors are encoded by genes located on plasmids or chromosomes. bacterial genome has been sequenced completely by the fictional of molecular technique (Suardana,2014). There is no enough information about genes that encode ferocity factors of *E.coli* and its role in

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urinary tract infection in Iraq, so that this research target to detect some ferocity genes by using molecular manner in *E. coli isolated* from UTI and study its roles in occurrence of UTI. ferocity genes that include in this study is *hly* (hemolysin), *cnf-1* (cytotoxic necrotizing factor), *ndvB* (biofilm formation) and *ure A* (urease).

II. MATERIALS AND METHODS

2.1 Bacterial strains and Identification

Five isolates of bacteria *E.coli* were proned in this research. Isolates were seized from patient with UTI in the June 2023 (from microbiology laboratory of Tikrit Military Hospital, Tikrit, Iraq). All isolates were grown on blood agar and macConkey agar plate at 37 ^{0}C for 18-24 hrs. Isolates were identified by colonial morphology on culture, gram stain and classical biochemical tests as described in (Brown and Smith,2015).

2.2 Extraction of DNA and PCR

A- DNA extraction:

The DNA of five *E.coli* isolates was extracted as described by the instruction of the promega kit.

B- Preparation the primers

The primers that includes in this research exposed in table (1).

Virulence factor	Target gene	Primer sequence	Size of amplicon (bp)	References
Haemolysin	*Hly*	F:GTA TTC GGC ACA GCA GAG AAA R:TTA ATG CTG GCA GCT GTG TC	323	(Al-Shammari and Hamza,2014)
Urease A	*Ure A*	F:GAC TCC AAG AGA AAA AGA CAA ACT A R:CAG ATT ATC GGA TTA TGG ACG GTA	100	(Friedrich et al.,2005)
Cytotoxic necrotizing factor	*Cnf-1*	F:AAG ATG GAG TTT CCT ATG CAG GAG R:CAT TCA GAG TCC TGC CCT CAT TAT T	498	(Yamamoto,1995)
Biofilm formation	*ndvB*	F:GGA CAG GGC AAG GTT TAT T R:GGT TAT ACT CAG CAG CAC TAT C	500	(Yamamoto,1995)

C- PCR amplification

PCR amplification was done for extracted DNA of bacteria in a perfect amount of 25 ul contented 5 ul template DNA, 0.5 ul of each of the primers, nuclease free water 6.5 ul, and master mix 12.5 ul, The PCR thermocycler program described in Table (2).

Table 2: program conditions for DNA amplification of *E.coli* genes.

Stage	Temperature C	Time	Cycle number
Initial Denaturation	95 C	5 min	1
Denaturation	95 C	40 sec	35
Annealing	X C	1 min	
Extension	72 C	40 sec	
Final extention	72 C	7 min	1

X, annealing temperature for each primer of *E.coli* virulence genes ; *cnf* -1 (58 C), *hly* (55 C), *ndvB* (57 C) and *ure A* (63 C).

PCR output were discreted on 1.5 % agarose gel with ethidium bromide and imagened beneath UV light, then photographed by digital camera.

III. RESULT AND DISCUSSION

E.coli remains a most troublesome health problem in different countries of the world. PCR is the useful technique for diagnosis and detected of virulence genes in pathogen. The current results showed that the frequency of the *cnf-1*, *hly*, ndvB and *ureA* genes in UTI isolated strains were 100% for each one (see fig 1-4). These results are consistent to various previous studies. Firoozeh, *et al*. (Firoozeh et al.,2014) found the genes coding for *hly* was 4% in patients with pyelonephritis and cystitis evolved by *E.coli*. While Alqasim *et al*. (Alqasim et la.,2020) from Riyadh, Saudi Arabia, found in study explore the genotypic traits of *E. coli* urine isolates, and provided comparisons among protracted spectrum β-lactamase – non-producing and producing *E. coli* isolates, they detected *hly* gene in 16.7% out of: 6(20%) in ESBL-producing and 4(13%) in non-producing *E. coli* isolates. In correlation to our presentment of results, Gazvini, *et al*., (Kudinha,2017), exposed a dissimilar frequency of *hly* with 12%, (Abdul-Ghaffar and Abu-Risha,2017) Abd El-Baky, *et al*., (Abd El-Baky *et al*.,2020) who found *hly* gene in (81.8%) of uropathogenic *E. coli* (UPEC).

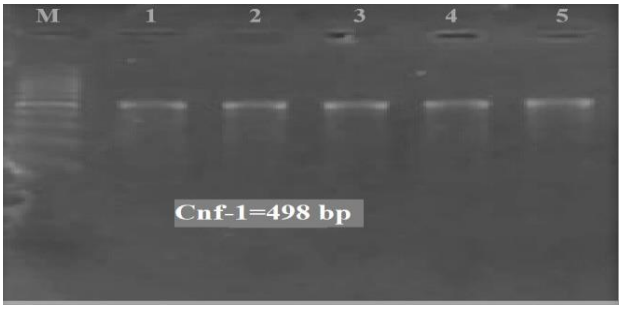

Figure 1. Amplification of Cnf-1 genes of *E.coli* by PCR. Lanes: M, 100 bp ladder marker, (1-5) clinical isolates were positive results for of Cnf-1 gene for *E.coli*

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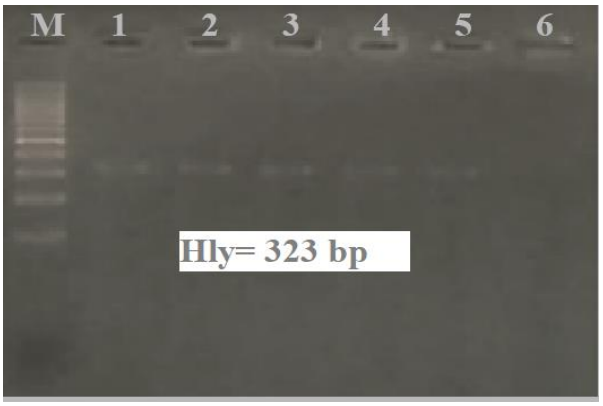

Figure 2. Amplification of Hly genes of *E.coli* by PCR. Lanes: M, 100 bp ladder marker, (1-6) clinical isolates were positive results for Hly genes of *E.coli*

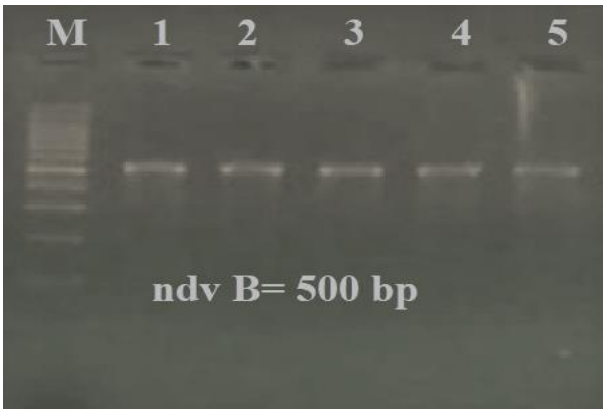

Figure 3. Amplification of ndv B gene of *E.coli* by PCR. Lanes: M, 100 bp ladder marker, (1-5) clinical isolates were positive results for ndv B gene of *E.coli*

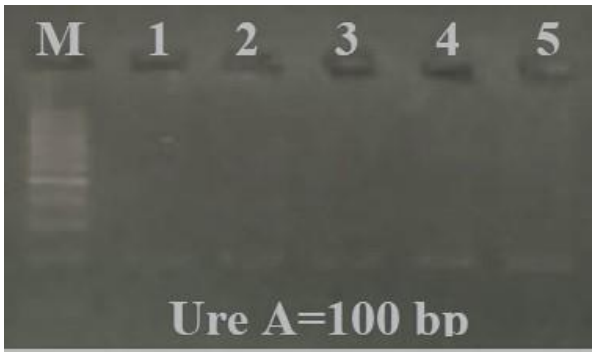

Figure 4. Amplification of Ure A gene of *E.coli* by PCR. Lanes: M, 100 bp ladder marker, (1-5) clinical isolates were positive results for *UreA* gene of *E.coli*

In Addis Ababa, Ethiopia, Dadi et al.,2020, found in his studies the *cnf-1* gene 58 (29 %) in isolates of *E.coli* among UTI patients. Uropathogenic *E.coli* exudate toxins like cytotoxic necrotizing factor *(cnf-1*) and haemolysin (*hly*). *Hly* involved in cell lysis by promotes exfoliation of bladder cell in patient and *Cnf* increased internalization of bacteria by also exfoliation of bladder cell.

Elshwehy *et al*.,2021, revealed that a total number of 13 samples were tested for biofilm formation gene expression; 10 (76.92%) of them were positive for *ndvB* biofilm formation from human urine samples. Mohammed *et al*.,2014 domenstrate in the *E. coli* strains the presence of *ndvB* gene in ten isolates (all isolates).

The findings in the current study are in consonance with the results that noted by Friedrich *et al*., 2005, Who establish *ureA* genes was presented in 58 of 59 (98.3%) Enterohemorrhagic *E.coli*.

The strains of *E.coli* with an extensive complement of virulence factors are more effective pathogens (Abdul-Ghaffar and Abu-Risha,2017).

The difference in prevalence of virulence factor between our study and different studies may be due to difference in sample size.

our results appearance the larger pervasiveness of ferocity factors in clinical isolates causing UTIs due to differences in social, health, economic, hygiene and environmental position between distinct countries, added studies are demanding to provide the pervasiveness of these and other ferocity factors in other local earth and to approve the relationship among ferocity factors and specific infections revealed in the current research. Furthermore, more studies are needed to determine the contact between the expression of ferocity factors and antibiotic resistance. These studies would be essential in understanding the role of these factors in UTIs, which in turn may lead to the evolution of global vaccines to avoid such infections.

IV. CONCLUSION

Urinary tract infections still the prevailing infection in the worldwide. The one of the most prevalent infectious diseases induce renal failure in patients is UTI that generated by UPEC. The presence of virulence factors are critical features where the degree of pathogenicities of UPEC strains dependent on it. This study indicate that was a real relationship among the ferocity factor genes of *E.coli* and clinical symptoms of UTI.

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