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ORIGINAL ARTICLE

MOLECULAR SCREENING AND DISTRIBUTION OF VIRULENCE GENES IN UROPATHOGEINC *E.COLI* ISOLATES AND ANTIBIOGRAM PATTERNS AMONG PATIENTS WITH UTI IN KARBALA PROVINCE

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ABSTRACT

Background: It has been determined that *Escherichia coli* is the prevailing uropathogen (50-90%) in both complicated and uncomplicated urinary tract infections. Uropathogenic *Escherichia coli* (UPEC) possesses an extensive array of virulence factors, which confers the potential to induce urinary tract infections and is correlated with the development of antibiotic resistance. The purpose of this research was to detect the distribution of virulence genes and their association with antibiotic resistance rates at the molecular level.

Materials & Methods: This study was conducted in the microbiology laboratory at Al-Hussein Medical city teaching hospital, Laboratory of microbiology in Karbala province, Iraq. Urine samples were collected b/w October 2023 to January 2024, and transported for further analysis using standard microbiological and biochemical techniques. The sampling technique used in the study can be described as convenience sampling. The identification of *E.coli* isolates was confirmed using 16rRNA, resistance and sensitivity to various antibiotics were determined with Vitek2 system. Identification of genes (*fimH*, *KpsmII*, *Pap*, *ompT)* by uniplex PCR.

Results: *E.Coli* was shown to be the most often isolated bacterium for various urine samples, with a percentage of 16(35.5%).The most virulent gene observed were *Fim H, KpsMII, pap and OmpT were*16 (100%), 13 (81.3%), 9(56.3%) and 8(50%) respectively. Thirteen type of antibiotics were identified sensitive to *E.coli* isolate the most common resistant rate was Ticarcillin, Pipraciilin had high resistance rate 14(87.5), Ciprofloxacin 9(56.3%), Minocycline, Aztreonam, Trimethoprim had rate of 8(50) . Sensitivity rate of Meropenem, Imipenem, Gentamicin was 14(87.5), Cefepime 13(81.25),Amikacin 12(75.0).

Conclusion: The study concluded that there is distribution of virulence gene in *E.coli* isolates , specially fimH,pap correlated with resistance to antibiotics specially with MDR and XDR patterns.

KEY WORDS: Antibiotic sensitivity; MDR; Uropathogenic *E.coli*; Virulence gene; XDR.

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1. INTRODUCTION

The most frequent pathogen diagnosed in renal and urological infections worldwide is *E.coli.*[1] Urinate elimination and blood purification constitutes the primary functions of the urinary system.[2,3] Microbes colonize and multiply in any urinary system organ, the body responds with inflammation, resulting in urinary tract infections (UTIs).[4] Urgency, dysuria, hematuria, frequent urination, and occasionally suprapubic pain have all been associated to these conditions.[5] For many years, urinary tract infections (UTIs) were the most common infection in several Middle Eastern and Iraqi population, due to a variety factors.[6,7] The Enterobacteriaceae family of bacteria is predominantly responsible for the majority of UTIs.[8] The most common strains of bacteria that cause UTIs are called uropathogenic *Escherichia coli* (UPEC), and they are responsible for around 95% of infections acquired in hospitals and communities as well as 80% of simple UTIs.[8] In order to cause long-lasting

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infections, UPEC strains display a number of virulence features, such as toxins, fimbrial and a fimbrial, adhesins, invasins, and systems for acquiring iron.[9] The flagellum, capsular lipopolysaccharide, and proteins of the outer membrane are further components of bacterial cell surfaces that contribute to their pathogenicity through attachment and colonization of the host cell and associated with biofilm formation and resistance to various types of antibiotics.[10-12] These virulence factors play a crucial role in allowing the bacteria to establish themselves in the urinary system and continue to thrive, even in the presence of a fully functional host defense mechanism and resistance to various types of antibiotics.[12-14] The objective of the current investigation was to ascertain the distribution of virulence factors and coronation with resistance rates to different types of antibiotics among *E.coli* isolates.

2. MATERIALS AND METHODS

2.1 Study design: Current cross-sectional study was conducted in the microbiology laboratory at Al-Hussein Medical city teaching hospital, Laboratory of microbiology in Karbala province, Iraq. From October 2023 to January 2024, urine samples were collected and transferred for further analysis using standard microbiological and biochemical techniques. The isolates of *E.coli* were then confirmed using 16rRNA.

2.2 Sample collection: The sampling technique used in the study can be described as convenience sampling.

2.2.1 Participants and Sample Collection:

- **Participants:** 100 patients with urinary tract infections (UTI) visiting Al-Hussein Teaching Hospital.
- **Sample Collection Process:**

> Urine samples were collected directly from patients using sterile containers.

> Samples were kept in a cool box to inhibit bacterial replication during transportation to the laboratory.

> Once at the laboratory, the samples were cultured on MacConkey Agar and incubated for 24 hours at 37°C.

> *E.coli* isolates were confirmed using 16S rRNA sequencing.

2.3 Diagnosis Using VITEK- 2 Compact System: Using the VITEK-2 system, selected *E.coli* isolate were chosen for identification and assessment of their susceptibility to antibiotics .

2.4 Genomic DNA extraction: On nutrient agar plates, identified *E. coli* isolates were streaked and left to incubate at 37°C for a full day. just one colony was chosen, and it was then placed in 50 mL of nutrient broth and incubated for nine hours at 37 °C on a rotary shaker spinning at 180 rpm. After that, the suspension was put into 1.5 mL sterile Eppendorf tubes so that DNA could be extracted. The procedure for extracting DNA was followed by the manufacturer (Presto™ Mini gDNA Bacteria Kit, Geneaid ,Taiwan). The determination of purity was conducted using a Nano-drop instrument manufactured by Analytik jena, Germany.DNA that had been extracted was kept at -20°C until tests called for it.

2.5 PCR assay: A uniplex polymerase chain reaction was utilized to amplify the genes *Pap, KpsmII,fimH , and ompT* for each isolate, and 16S rRNA genes were employed to confirm the isolate. The details of the primers used in the study is given in (Table 1). A final volume of 25 μL was utilized for polymerase chain reaction (PCR), and the resulting products were analyzed by separating them on a 1.5% agarose gel to confirm their expected sizes.

Table 1: Displays the primers that were utilized for PCR amplification.

N.	Primer Name	Sequence	Product size (bp)	Reference
1	**kpsMII-F** **kpsMII-R**	5`-AAGTCAAAGCAGGGGTTGCCCG-3` 5`-GACGCCGACATTAAGACGCAG-3`	668	[15]
2	**pap-F** **pap-R**	5`-GACGGTGTACTGCAGGGTGTC-3` 5`-ATATCCTTTCTGCAGGGATGCAA-3`	328	[16]
3	**ompT-F** **ompT-R**	5`-ATCTAGCCGAAGAAGGAGGC-3` 5`-CCCGGGTCATAGTGTTCATC-3	559	[17]
4	**fimH-F** **fimH-R**	5`-AACAGCGATGATTTCCAGTTTGTGTG-3` 5`-TTGCGTACCAGCATTAGCAATGTCC-3`	465	[16]
5	***16SrRNA***	5`-AGAGTTTGATCCTGGCTCA-3` 5`-GGTTACCTTGTTACGACTT-3`	1400	[18]

3. RESULTS

3.1 Prevalence of *E.coli* in human urine infection: Out of 100 samples collected from patient infected with UTI from Al-Hussein Medical city teaching hospital /Kerbela/Iraq. Forty five samples was positive for different type of bacteria , *E.coli* was 16(35.55%) as shown in Figure 1.

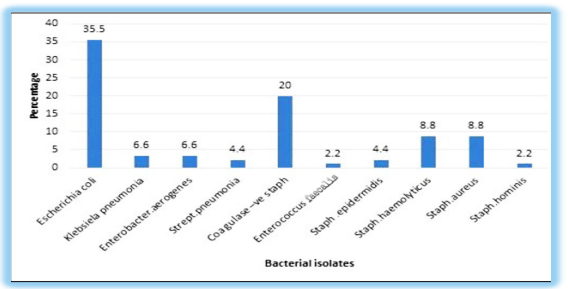


Figure 1: Uropathogenic bacteria profile among patients infected with UTI

3.2 Molecular detection of fimH, KpsmII ompT pap genes and 16SrRNA: Figure 2 illustrates the uniplex PCR result for 16 (100%) *E.coli* isolates that complied with the 16srRNA gene detection at a product size of 1400bp. Additionally, all *E.coli* isolates tested positive for the Fim H gene amplification at a product size of 450bp, as shown in [Figure 3]. Figure 4 illustrates the distribution of the KpsmII gene, which was detected in 13 (81.3%) of the clinical isolates of *E. coli,* using an amplification product of 668bp. The pap gene was detected 9 times (56.3%), resulting in a product size of 465bp [figure 5]. The OmpT gene was found 8 times (50%) in the *E. coli* isolate, with a product size of 595bp [figure 6]. The percentage of virulence factors for *E. coli* is depicted in Figure 7.

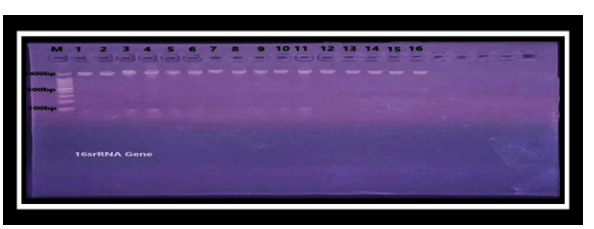
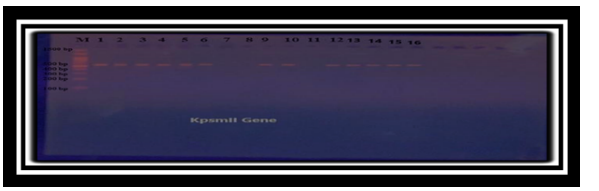
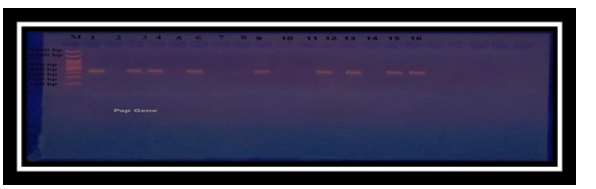


Figure 2: PCR amplification of 16srRNAGene 1% Agarose gel electrophoresis(75volt ,for 1 hrs),all isolates had 16srRNAGene .

Figure 3: Electrophoresis of amplified fimH (465bp).Agarose gel 1%,75volt for 1hrs,all isolate had fimH.



Figure(4): PCR amplification of KpsmII gene ,product size(668bp)13 sample was positive except (7,8,11)



Figure(5):PCR amplification of Pap gene with the product size (328bp). Isolates 1,3,4,6,9,12,13,15,16 positive result,isolate2,5,7,8,10,14,11 negative results.

Figure (6) :Electrophoresis of amplified ompT (559bp) .Agarose gel 1%,75volt for 1hrs. Isolates 1,3,5,8,10,12,13,15 positive result,isolate2,4,7, 6,9,14,16 negative result.

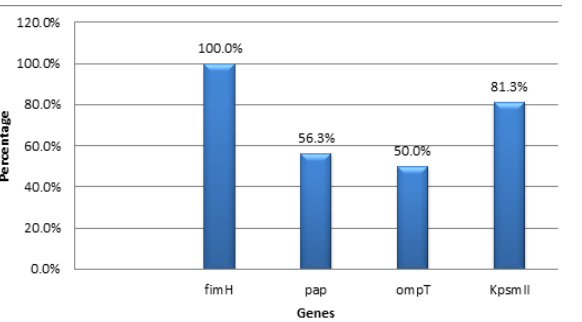


Figure (7): distribution of virulence factor isolate among *E.coli* isolates from UTI

3.3 Antibiotic susceptibility of Escherichia coli isolates: A test for the antibiotic susceptibility of 16 UPEC isolates was performed using Vitek2 system. The isolates in this investigation varied significantly in how they responded to the antibiotics utilized, as seen in [Table 2] .The profile resistance rate for different type of antibiotics revealed that Ticarcillin, Pipraciilin was 14(87.5%), Ciprofloxacin was 9(56.3%), Minocycline ,Aztreonam and Trimethoprim was 8(50.0), the greatest susceptibility was seen to Meropenem, Imipenem, Gentamycin was 14(87.5%),Cefepime was 13(81.2%).

Table 2: Antibiotic susceptibility for *E.coli* isolates

Antibiotics	Susceptible N	(%) Resistant N (%)
Ticarcillin	2(12.5)	14(87.5)
Pipraciilin	2(12.5)	14(87.5)
Ceftazidime	11(68.8)	5(31.3)
Cefepime	13(81.25)	3(18.75)
Meropenem	14(87.5)	2(12.5)
Imipenem	14(87.5)	2(12.5)
Tobramycin	12(75.0)	4(25.0)
Gentamicin	14(87.5)	2(12.5)
Amikacin	12(75.0)	4(25)
Ciprofloxacin	7(43.8)	9(56.3)
Minocycline	8(50.0)	8(50)
Aztreonam	8(50.0)	8(50.0)
Trimethoprim	8(53.3)	8(50.0)

In the this study conducted that PCR findings indicated two isolates (E11,E7) had 1 virulence gene ,3 isolate (E2,E8,E14) had 2 virulence genes,6 isolate (E4,E5.E6,E9,E10,E16) had 3 virulence genes, and 4 isolate (E1,E3,E12,E13,E15) had all of the virulence genes as shown in [table 3] many isolates have a resistance like (XDR,MDR or None) .

4. DISCUSSION

Over 90% of urinary tract infection are caused by gram negative bacteria especially *E.coli* and both nosocomial and community acquired urinary tract infection.[2,16] There are many virulence gene associated with UTI and responsible for resistance for antibiotics like FimH , ompT and other genes associated colonization and adhesion then with biofilm formation.[19] The current study revealed that bacterial prevalence among 100 sample collected was 45 only ,the highest percentage of identified was *E.coli* 16(35.5%) this study is enrolled with results[20] that prevalence of *E.coli* in UTI patient was(34.75%). The distribution of virulence gene associated with UTI was 16(100%) belong to FimH gene then KpsmII ,pap and ompT was 13 (81.3%),9(56.3%),8(50%) respectively that enrolled with studies[21] Jalwla'a and Khanaqin General Hospitals. This included pregnant and non-pregnant women with an age range of 15 – 40 years from eastern Diyala Province during the period from September to November. Primary diagnosing of the isolates was done by specific culture media (MacConkey agar, Eosin-Methylene Blue, blood agar[22-24],where the percentage of genes in these studies were (100%),(76%),(57%),(54%) respectively. The proportion of resistance that was greatest among the E. coli isolates was documented against Ticarcillin (87.5%), Pipracillin (87.5&).Allami et al (2022) Iraq, who discovered that Ticarcillin

Table 3: Genetic patterns and types of resistant of study isolates

Bacterial code	No. of genes	Gene detected	Types of resistant
E1	4	FimH,KpsmII,OmpT,Pap	*XDR
E2	2	FImH,KpsmII	XDR
E3	4	FimH,ompT,Pap,KpsmII	* NON
E4	3	FimH,Pap,kpsmII	MDR
E5	3	FimH,ompT,kpsmII	NON
E6	3	FimH,pap,kpsmII	XDR
E7	1	FImH	MDR
E8	2	FimH,ompT	NON
E9	3	FimH,pap,KpsmII	NON
E10	3	FimH,ompT,KpsmII	* MDR
E11	1	FimH	NON
E12	4	FimH,ompT,pap,KpsmII	XDR
E13	4	FimH,KpsmII,OmpT, Pap	XDR
E14	2	FImH,KpsmII	NON
E15	4	FimH,ompT,Pap,KpsmII	MDR
E16	3	FimH,Pap,kpsmII	NON

* XDR= extensively-drug resistant, MDR= multi-drug resistant, NON =no have pattern

resistance were (92%, 91%) are consistent with the current investigation.[25] The moderate percentage of resistance among the *E.coli* isolates was recorded against ciprofloxacin(56.3%) ,Tajbakhsh et al in Iran (2016) who discovered that ciprofloxacin resistance was 56.25%, are consistent with the current investigation.[26] It was demonstrated that the bacterial biofilm development was the cause of the developed resistance to ciprofloxacin.

The percentage rate of resistance was recorded against Ceftazidime 11(68.8%), the result of agrees with study done in Iran where they found the sensitivity rate to ceftazidime 281(82%), are consistent with the current investigation.[27] Study in Iraq Ahmed *et al.*[21] who discovered that resistance to ceftazidime recorded (86.6%), which disagree with our study, and not enrolled with study in Iraq (2022) by Mahde *et al* that found the high degree of resistance was 62 (87.32) to ceftazidime [28]. The lowest percentage of resistance was recorded against cefepime(18.75%),the result of Tarverdi et al., (2024) which agreed with our study[29] . With a wide range of antibacterial action, carbapenems are the most effective beta-lactam antibiotics against microorganisms that are Gram-negative and Gram-positive[30] .*E. coli* strains with the highest levels of sensitivity was recorded to carbapenems group (meropenem 87.5% and imipenem 87.5%), the result of [31] they found the sensitive to carbapenems group was recorded (meropenem ,imipenem was 100%) are consistent with the current study .

The study identified *E.coli* isolates that exhibited resistance patterns (MDR, XDR) to the antibiotics that were tested, with a rate of 50% and distribution of gene may related to the resistance rate like FimH , pap that related to biofilm formation and Outer membrane protein that associated with crucial function like transportation of antibiotics, iron and host mucosal adhesion.[32] Boroumand et al [33] found there is strong correlation between resistance and FimH gene that is consist with current study by Ghavidel et al.[34] It was found that there is correlation between pap gene and antibiotic resistance among participant that enrolled with our study. According to the study's findings, the majority of isolates were MDR, and this could be because the cells had acquired a plasmid or had a mutation that increased their level of resistance. Numerous E. coli strains that are resistant to multiple drugs have emerged as a result of the extensive and pervasive application of broad-spectrum antibiotics.

5. CONCLUSION

The common antibiotics sensitive for UTI treatment (Meropenem, Imipenem, Gentamicin) are used for treatment of UTI infected by E.coli .There is relation between FimH pap, Omp resistance rate for different type of antibiotic with MDR and XDR. The resistance rate was observed in E7,E10,E13 of *E.coli* multidrug resistance for four antibiotics ,E4 resistance to 3 antibiotics , E1,E12 resist to 7 type of antibiotic called Extensively drug resistant ,E2 belong to XDR.

Ethical consent: The study protocol, subject information, and consent form underwent review and approval by the ethical committee of the University of Karbala / College of Medicine, as documented by reference number 31. Additionally, the Karbala Health Directorate and ALHussein Medical City provided their consent for the study.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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AUTHORS' CONTRIBUTION

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All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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