# A Study On Some Virulence Factors And Its Relation To Drug Resistance Of Escherichia Coli Isolated From Urinary Tract Infection

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## Abstract:

Urinary tract infection (UTI) is a bacterial infection which can happen anywhere along the urinary tract, it is a common health problem in the world, affecting both sexes with different ages. Effective therapy is based on antibiotics, but bacterial resistance is an ongoing issue for management of UTI, there is a serious health threats with respect to drug resistance. This study aimed for focusing the relation of drug resistance and some virulence factors of the main UTI causative agent, Escherichia coli, which was conducted in Baghdad hospital. 160 mid stream urine samples were collected from patients, and processed, to detect virulence factors of UPEC strains isolated and to compare the frequency of drug resistance pattern. The results revealed higher proportion of UTI in females than in males. E. coli isolates were tested against ten antibiotics. Their effect ranged from resistance to intermediate susceptibility to full sensitivity. The results showed that all E. coli strains were highest rate of resistance with Amoxicillin, Amoxicillin/clavulanic acid and moderately resistant to Cerfotaxim, CoTrimoxizole (Trmiethoprim-Sulfacmehaxazole), levofloxacin and Nadixic acid, where as some isolates had shown lowest rates of resistance to Nitrofurantion, Ciprofloxacin, Norfloxacin and mipenem. E. coli showed multiple resistances to more than one antibiotic, a collective result obtained showed that 17 (65.4%) strains from a total of 26 E.coli isolated from urinary tract infections, while isolates with low resistance is 9 (34.6%.).

دراسة عن بعض عوامل الضراوة وعلاقتها بمقاومة الادوية لجرثومة الايشيريشيا القولونية المعزولة من حالات التهاب المجاري البولية

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#### ملخص البحث:

إن التهاب المجاري البولية مرض جرثومي شائع ومشكلة صحية في الكثير من المجتمعات في معظم إرجاء العالم ويصيب أي جزء من أجزاء المجاري البولية في كلا الجنسين وبين كافة المراحل العمرية و يتم علاج حالات التهاب المجاري البولية باستخدام المضادات الحيوية إلا أن مقاومة الجراثيم المعوية المسببة للمرض تعقد حالات الشفاء من هذا المرض فأصبحت حالات المرض مما يسجل يوميا في المستشفيات.

تهدف هذه الدراسة الى القاء الضوء على علاقة بعض عوامل الضراوة للجرثومة الرئيسية المسبببة للمرض الايشيريشيا كولاى والمقاومة للادوية المستخدمة فى علاج الحالات من المضادات الحيوية ٠ وقد اجريت الدراسة في مستشفى بغداد التعليمي وبعض المستشفيات الخاصة حيث جمعت 160 عينة ادرار من المرضى تتراوح اعمارهم بين 5–15 عام وتم معالجة العينات الماخوذة حسب الطرق المعتمدة وتم تشخيص العزلات الجرثومية بالاختبارات البيوكيمياوية اللازمة وفحص الحساسية للعزلات الجرثومية ومعاملتها مع المضادات الحيوية اضهرت النتائج ان نسبة الاصابة بين الاناث اكبر من نسبة الاصابة بين الذكور وتراوحت النتائج بالنسبة للادوية بين المقاومة الكاملة و المقاومة المتوسطة، نستنتج من هذه الدراسة بان نسبة الإصابة بحالات الالتهاب في المجاري البولية كبير بين افراد المجتمع وهذه تستدعي ان يقف الكادر الصحي على الأسباب المؤدية لذلك ونشر الوعى الصحى والاعتناء بالحالات المرضية التى تسببها جرثومة الاشيريشيا القولونية وتجنب سوء استخدام المضادات الحيوية

#### Introduction:

Urinary tract infection (UTI) is an infection involving part or all of the urinary tract which remains a common and troublesome health problem in many different countries all rounds the world (1). In most hospitals a very large numbers of UTI cases recorded daily among different ages and sexes, these infections may involve the urethra, bladder, ureters, the kidney and prostates, the extent of infection depends on the interaction between the bacteria and host's defense mechanisms, an infraction can occur anywhere along this tract, but the lower parts of the urethra and bladder are most commonly involved, this is called cystitis, if the infection travels up the ureters to the kidney it is called pyelonephritis; about 75% to 90% the causative agent of these complication are E.coli. (2,3)

The extensive uses of antimicrobial agents have invariable results in the development of antibiotic resistance, which in recent years, has become a major problem worldwide. (4)

The virulence factors of uropathogenic E.Coli (UPEC) are mainly responsible for the severity of these emerging infections (5). In humans, the virulence factors help for bacterial colonization in urinary epithelium and cause severe acuity in (UPEC) strains which is responsible for causing severe diseases and resistance against drugs (6;7).

#### Aims and objectives

The study is essential in detection of some virulence factors of UPEC strain isolated from patients with UTIs and to compare the frequency of drug resistance pattern in E. coli isolated from patients with clinical UTI.

#### Materials and Methods:

Samples: A total of 160 E coli from symptomatic cases of urinary tract infection attending outpatient of Baghdad Educational Hospital and some private hospitals at Baghdad . For the period between Jan. to June 2011. Mid stream urine samples were processed according to standard protocol (8). Lactose fermenting colonies on MacConkey's agar (MA) showed significant bacteria in urea, samples were processed and identified, the bacteria were identified as E.coli by standard biochemical tests (9) And confirmated by api20 kit (10).

## Results and discussion:

Isolation of Escherichia coli: One hundred sixty urine samples were collected from urinary tract infection patients; with ages ranged from 15 to 50 old were examined in this study. All the patients were hospitalized, and suffed from one or more of UTI symptoms, UTI diagnoses were established by the hospital medical staff based on clinical symptoms and laboratory investigation. Urinary frequency, internal dysuria and suprapubic or pelvic pain are the characteristic symptoms of cystitis, in general acute pyelonephritis presents fever, flank pain, nausea and vomiting. Table 1 shows demographic data of UTI patients.

Table 3-1: D	emographic data	of UTI patients.
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Variable	Data	
Age of patients	15-50 years	
Sex of patients	103:47	
Female : male	105.47	
Clinical case in adult women UTI	79 cases	
Clinical case in girls UTI	34 cases	
Clinical case in men UTI	47 cases	

This table indicates that a higher proportion of UTI in females were more than in males. This is understandable due to the anatomy and is a consistent trend worldwide. The study is agreement with Jadhav et.al., (2011).(11)

Only 26 isolates of E . coli among these clinical samples were isolated. These compatible with (12,13) which they found that E.coli is the commonest cause of urinary tract infection in women and children especially in those with uncomplicated infections.

Table 3. The relationship between age ratio and positive Num-
ber of UTI & E.coli in pregnant women

Age group (year)	Age ratio	Number of positive (%) UTI patients	Number of positive (%) E.coli
15-20	17.5	25 (25.5%)	5 (20%)
21-25	23	38 (38.7%)	9 (23.6%)
26-30	28	15 (15.3%)	6 (40)
31-35	33	15 (15.3%)	2 (13.3)
36-40	38	3 (3%)	2 (66.66)
41-50	45.5	2 (2%)	1 (50%)

These were investigated for possession of virulence factors. All isolates had one or the other virulence factors. These finding agree with Moreno et al. (2008) (14)\_which found that E. coli strains responsible for UTIs are characterized by faecal abundance, a higher virulence factor score and to be of phylogroup B2 origin in comparison with those strains found in faucal samples only.(14) Haemolysin production was observed in 6 (23%) of uro isolates. The result similar to Kausar et al. (2009) result which noticed among 200 Escherichia coli 42(21%) were haemolytic. (12)

Whereas adherence of E.coli isolates to uroepithelial cells is used were showed that 100% of the isolates have this ability. The result of adherence to uroepithelial cells agree with Gupta et al., (2011). (15)

All E. coli isolates were tested against the ten antibiotics. Their effect ranged from resistance to intermediate susceptibility to full sensitivity (Figure1). The results showed that all E. coli strains were highest rate of resistance is seen with Amoxicillin, Amoxicillin/clavulanic acid and are moderately resistant to Cerfotaxim, CoTrimoxizole (Trmiethoprim-Sulfacmehaxazole), levofloxacin and Nadixic acid where as some isolates have shown lowest rates of resistance to Nitrofurantion, Ciprofloxacin, Norfloxacin and trimethoprime.



Fig. 1: Percentage of antibiotic resistant E.coli isolates from UTI patients

E. coli showed multiple resistances to more than one antibiotic, a collective result obtained out of table (3) indicates that 17 (65.4%) strains from a total of 26 E.coli isolated from urinary tract infections, while isolates with low resistance is 9 (34.6%). The prevalence of MDR among clinical isolates very greatly worldwide and in geographic areas and are rapidly changing over time . This study is agreement with Zhanel et al.(16).



Fig. 2 : PCR Agarose gel electrophoresis (1% agarose, 7 v/ cm2) and Ethidium bromide staining to detect sfaDE genes size products (410bp) respectively using template DNA prepared by boiling method. Lenc5M, molecular size DNA ladder (123 bp DNA Ladder).



Fig. 3 : PCR: Agarose gel electrophoresis (1% agrose, 7 v/cm2) and Ethidium bromide staining to detect papC genes size products (bands 328bp) respectively using template DNA prepared by boiling method. Lenc5M, molecular size DNA ladder (123 bp DNA Ladder).

#### Table 4 : Multi- drug resistance of UTI E.coli

Groups	No. of antimicrobial which resisted by isolates	No. of the multidrug resistance isolates	Percentage of multidrug resistance
А	0-5	9	34.6%
В	6-10	17	65.4%

#### **Conclusion**:

There are higher proportion of UTI cases in females than in UTI in males.

E. coli isolates were tested against ten antibiotics. Their effect ranged from resistance to intermediate susceptibility to full sensitivity.

## **Recommendations:**

An intensive health education programme is needed about this important issue of increase UTI cases in the community, including causes preventive measures ant treatment and implementation of this program periodically in different areas to increase awareness of people .

Avoid Antibiotics abuse, increase awareness regarding drugs used to treat UTI cases, the right dosage, for the right time.

Adopt healthy life style, hygienic practices, increase of pure water intake

Further studies are recommended in different areas

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#### **References**:

- Antony Y. Peleg and D.C. Hooper: Hospital Acquired Infections due to Gram-Negative Bacteria. N.Engl.J.Med. 2010; 362: 1804-1813.
- Nicolle L.E. Epidemiology of UTI. Infect. Med. 2001; 18:153-156
- Gilbert DN .Urinary tract infections in patients with chronic renal insufficiency. The American Society of Am Soc Nephrol. 2006; 1 (2) :327-331.
- A. Karimian; Momtaz H. and Madani M. Detection of Uropathogenic E.coli virulence factors in patients with UTIs in Iran. African J. of Microb. Research. 2012; 6(39): 6811-6816.
- Bashir Abul Haque; Y. Saruar; A.Ali and M. Anwer: Annals of Clinical Microbiology and Antimicrobials 2012; 4: 23.
- Silveria, W; Beneti F.; Lancelotti ; Ferriera A.; Solferini, V. and Brocchi, M. Biological and genetic characteristics of UPEC strain. Rev. inst. Med. Trop. Sao Paulo. 2001; 43:303-310.

- Kumer M.S, Lakshmi V. and Rajagopalan R. spectrum beta lactamases among Entrobacteriaceae spp isolated at a tertiary care institute. Ind. J. Med. Microbiol. 2006; 24: 208-21 Occurrence of extended.
- Kunin, C. M. Urinary tract infection and patient. In: cecil textbook ofmedicine. 20th ed. .Philadelphia: W.B. Sanders Co.1996. Pp: 602 – 605.
- Chessbrough, M. Collection, transport and examination of specimens. In: Chessbrough, M. (Ed). Medical laboratory Manual for Wort-HeinemannLtd, Oxford. 1993.
- CLSI, (Clinical & Laboratory Standards institute).Performance standard for antimicrobial susceptibility testing; Seventeenth informational supplement. M100-S17. 2007; 27(1).
- Jadhav, S. Hussain, A., Devi, S., Kumar A., Parveen S., Gandham N., Wieler, L.H., Ewers C.& Ahmed, N. Virulence Characteristics and Genetic Affinities of Multiple Drug Resistant UropathogenicEscherichia coli from a Semi Urban Locality in India. PLoS. 2011; 6(3).
- Kausar, Y., S.K. Chunchanur, S.D. Nadagir, L.H. Halesh and M.R. Chandrasekhar. Virulence factors, serotypes and antimicrobial suspectibility pattern of Escherichia coli in urinary tract infections. Al Ameen J. Med. Sci. 2009; 2: 47-51.
- Pallett A., K.Hand .Complicated urinary tract infections;practical solutions for the treatment of multiresistant Gram-negative bacteria. J.Antimicrob.Chemother. 2010; 65: 25-33.
- Moreno E., Andreu A., Pigrau C., Kuskowski M. A., Johnson J. R., Prats G. Relationship between Escherichia coli strains causing acute cystitis in women and the fecal E. coli population of the host. J Clin. Microbiol. 2008;46: 2529–2534.
- Gupta A., Dwivedi M., Mahdi AA., Nagana GA., Khetrapal GL., Bhandari M., Inhabtion of adherence of multi – drug resistant E.coli proanthocyanidin .Urol. Research . 2011; 40 (2): 143-150
- George G. Zhanel ;Tamiko L. Hisanaga and NM Laing . International journa of antimicrobial Agents. 2006 ; 27(6) :468-475.