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Prevalence of resistance E.coli against trimethoprim and sulfmethoxazole in patients with UTI

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Abstract

Clinical isolates of *Escherichia coli* (35 isolates) were obtained from patients suffering from UTI from Baghdad Medical City hospital during the period between 1st December/2015 till 1st April. The isolates were identified by culturing on MacConkey agar and blood agar then diagnosed by performing some morphological and biochemical tests. In this study 22/35 (62.8%) of the sample were isolates from female and 13/3 (37.1%) isolates from male. Antibiotic susceptibility test for two antibiotics was performed using disc diffusion method. The percentage of highest resistance in *E.coli* isolates reached 28.5% (10/35) for ciprofloxacin while lower reached 14.2% (5/35) Trimethoprim and sulfamethoxazole. Detect the resistance rates according to the sex of the patients showed significant differences for resistance to Cip in male 17.1% (6/35) compared with 14.2% (5/35) in female and for Co-tri in female 5.7% (2/35) compared with 8.5% (3/35) in male.

INTRODUCTION:

Urinary tract infections (UTIs)

Urinary tract infections (UTIs) are some of the most common problems seen both in community as well as in hospital settings [1]. UTI may involve only the lower urinary tract (UT) or both the upper and the lower tracts [2]. The urethra and urinary bladder are the most frequent sites of infections within the UT [3]. UT successful invasion depends on the bacteria virulence, inoculums size, and the host's defense mechanisms [4,5].

Prevalence of UTI in women:

Every woman has approximately 60% lifetime risk of developing bacterial cystitis, which develops mostly before the age of 24. By contrast, men have a lifetime risk of only 13% [6]. In many cases, women is managed effectively and safely by empirical antibiotic therapy without the need for a urine culture [7]. Therefore, physicians have been informed that empirical antibiotic treatment without culture is convenient in such cases. The empirical therapy has been so widely used that only a few UTIs are routinely cultured [8]. Worldwide data shows that there is an increasing resistance to conventional drugs among UTI pathogens [9,10]. So UTI treatment may vary according to patient age, sex, Pathogen involved, course of disease, and the urinary tract anatomic area involved [5].

Causative agent of UTI:

UTIs are predominantly caused by bacteria. The most common bacteria implicated as causative agents of UTI generally originate in the intestine and include but not limited to *E.coli. Pseudomonas spp, Proteus spp., Klebsiella spp., Staphylococcus spp., Streptococcus spp, Neisseria gonorrhoea, Chlamydia trachomatis, Candida spp., Mycoplasma* are also other causative organisms [10]. However, to our knowledge it is *E.coli* one of the pathogenic agents of UTI and it is more frequent in women, with high incidence and prevalence, representing a costly problem for the health sector [5,11,12].

Escherichia coli

Escherichia coli bacteria belong to family Enterobacteriaceae, a large heterogeneous group of Gram negative rods whose natural habitat is the intestinal tract of human and animal [13]. This type of bacteria were named by The odor Escherich, who isolated the type species of the genus [14]. *E.coli* is one of the most intensively studied living species, while it has been widely used in experimental studies of evolution [13]. This bacterium is straight, cylindrical Gram negative rods with rounded ends and that are 1.1-1.5 µm in diameter and 2.0-6.0 µm in length, occur singly or in pairs and either motile or non-motile and are non-spore forming bacteria [15].

This bacterium can quickly identify as *E.coli* by its β-hymolysin on blood agar media and its typical colonies with iridescent "sheen" on Eosin Meythylene Blue (EMB) agar. Grow well on McConkey agar and form circular, convex, smooth colonies with distinct edges beside giving pink colour as induction for lactose fermentation [16]. *E.coli* strains survive and grow over the midrange 15-45˚C [14], nevertheless, Fotadar *et al.* [17] mentioned that *E.coli* can grow consistently at a temperature as high as 49˚C. *E.coli* consider a natural and essential part of the bacterial flora in the gut of human and animals [14,15]. It is the cause of many diseases, ranging from minor disease to severe life threatening sepsis [18]. This normally commensal need only acquired a combination adapted elements to become highly adapted pathogen capable of causing a range of diseases, from gastroenteritis to extra intestinal infection of urinary tract, bloodstream and central nervous system [19].

Clinical importance of *Escherichia coli*

E.coli strains causing UTI are termed uropathogenic *E.coli* (UPEC). UPEC isolates are a genetically heterogeneous group that possess several virulence factors (VFs) necessary for persistence and colonization of the bacteria in the urinary tract, overcome host defenses, and extra intestinal disease [5,20]. The virulence of individual strains in a given infection is determined by the presence and actual expression of the virulence genes present in them and also by the environmental conditions in the host. The virulence

factors of *E.coli* include ability to adhere to uroepithelial cells, haemagglutination, serum resistance, haemolysin, cell surface hydrophobicity, resistance to phagocytosis, cytotoxic necrotizing factor, K1 antigen, siderophore and gelatinase production and others [21, 22]. Antimicrobial resistance in *E.coli* has been reported worldwide and increasing rates of resistance among *E.coli* is a growing concern in both developed and developing countries [18].

There are a large group of antimicrobial agents available for the treatment of among extra intestinal *E.coli* caused UTIs [22] and, often require more complicated treatment regimens. The evolution of antimicrobial resistance in community acquired Escherichia coli, however, requires continuing reevaluation of empiric antimicrobial therapy [23]. For instance, in 2001 , Manges *et al* [24] were report that *E.coli* caused 11% of all *E.coli* UTIs and 49% of all trimethoprim/sulfamethoxazole-resistant *E.coli* in UTIs in California community over a 4-month period. It caused antimicrobial drug–resistant UTIs in Michigan, Minnesota, and Colorado [25], and pyelonephritis in several states [26,27].

Antibiotics of this study:

1. **Trimethoprim-sulfamethoxazole** (160/800 mg [1 double strength tablet] twice-daily for 3 days) (Co-tri):
   Trimethoprim and sulfamethoxazole have a greater effect when given together than when given separately, because they inhibit successive steps in the folate synthesis pathway.
2. **ciprofloxacin (1000 mg extended release for 7 days) (cip):**
   It is an appropriate choice for therapy in patients suffering from Complicated urinary tract infections and pyelonephritis due to *E.coli*, [28] but never as first-line agents. Current recommendations by the American Academy of Pediatrics note the systemic use of ciprofloxacin in children should be restricted to infections caused by multidrug-resistant pathogens or when no safe or effective alternatives are available [29]. Also use to patients not requiring hospitalization where the prevalence of resistance of community uropathogens is not known to exceed 10% [30]. In some cases use ciprofloxacin in combination with metronidazole is one of several first-line antibiotic regimens in treatment guidelines for acute pyelonephritis, complicated or hospital-acquired urinary tract infection [31].

MATERIALS AND METHODS

Antibiotic discs

All from Bioanalyse (Turkey) company.

Antibiotics	Code	Concentration µg/disk
Trimethoprim-sulfamethoxazole	Co- Tri	30 µg
Ciprofloxacin	CIP	10 µg

Sterilization methods

Moist heat sterilization was used to sterilize media and some solutions that are not affected by heating, using autoclave under 15 bar/in² pressures at 121 °C for 15 minutes, while dry sterilization was used to sterilize glassware at 160-180 °C for 2-3 hrs.

Preparation of cultural media

Readymade media including: Eosin methylene blue agar (EMB), MacConkey agar and Muller Hinton agar and Laboratory prepared media including Blood agar were prepared according to the instructions of the manufacturing companies.

Samples collection

Thirty five of *Escherichia coli* isolates were obtained from patients suffering from UTI from Baghdad Medical City hospital during the period between 1st December/2015 till 1st April.

The urine samples taken by mid-stream types then culturing on EMB agsr , MacConkey and blood agar to diagnose these samples by morphological and biochemical test. The bacterial diagnosis as *Escherichia coli* were tested for antimicrobial susceptibility depending on the CLSI [32].

All patients in this study take data about them from hospital records such as, age and sex of them.

RESULTS AND DISCUSSION

Collection and Diagnosis of the isolates:

In this study, thirty five isolates were diagnose as *Escherichia coli* collected from patients suffering from urinary tract infections. The Infectious Diseases Society of America has recognized Gram-negative pathogens as the most problematic bacterial challenges in the infectious disease community [33]. It represents the most frequent pathogens especially in hospitals environment causing serious problem for their widely distribution [34].

The 35 collected isolates were diagnosed as *E.coli* cause UTI. To confirm this diagnosis, the bacterial isolates preliminary cultured on MacConkey agar and blood agar in aerobic conditions followed by other differential diagnostic tests. On MacConkey agar, *E.coli* appeared pink in color due to lactose fermentation and small in size with regular edge. On blood agar, some isolates produced a clear translucent area surrounding the colonies as a result for β-hemolysine production, while the growth on EMB agar appeared as black colonies with greenish-black metallic sheen [35]. Microscopic observation showed these bacteria as Gram negative rods arranged signally or aggregates in pairs.

E.coli was diagnosed (in hospital) as a positive result in the catalase test, while it gave negative results in oxidase and urease. For IMViC test, it gave a positive results in indol test and methyl red test, while a negative result appeared with voges proskauer besides it was unable to utilize citrate as a main source for carbon(table1). Culturing *E.coli* in TSI media showed that this bacteria produced acidic reaction on the slant and the bottom with gas production due to the fermentation of the three sugars

(lactose, glucose, and sucrose), with no H_2S production [15].

Table (1): The results of some biochemical tests for *E.coli*

Biochemical tests	*E.coli*
Oxidase test	-
Catalase test	+
Indol test	+
MR test	+
testVP	-
Citrate utilization test	-
TSI test Slant/bottom H_2S Gas production	 A/A - +
Urease test	-

+: Positive result, **- :** Negative result, **A:** Acid production, **K:** Alkaline, **NG:** No change.

So it could be said that *E.coli* comprises the major cause of UTI as compared with the others bacteria and about 90% of cases is correlated with these bacteria as AL-Gerawi [36] had mentioned in their research. This is may be due to that UT is a common for invasion of various gram negative pathogens and this finding was also observed by Al-Jubori, [37] who ensured that UTIs was among the most commonly prevalent infections by *E.coli* pathogens in clinical practice. Thus, the current study only *E.coli* was isolated from patients suffering from UTI.

Correlation between UTI and sex of patients

The 35 *E.coli* urinary isolates were from 22 female and 13 males (range 29-80 years and only one female with age 2 years). The result of the current study comparable with the results of Alo´s *et al*. [7] who identified 164 isolates of *E.coli* in which 68.2% of female and 31.7% male. But Jain *et al.*, [38] was seen *E.coli* is common in both female (65.09%) as well as in male (67.25%). So can be say that UTI is more common among women than men in this study.

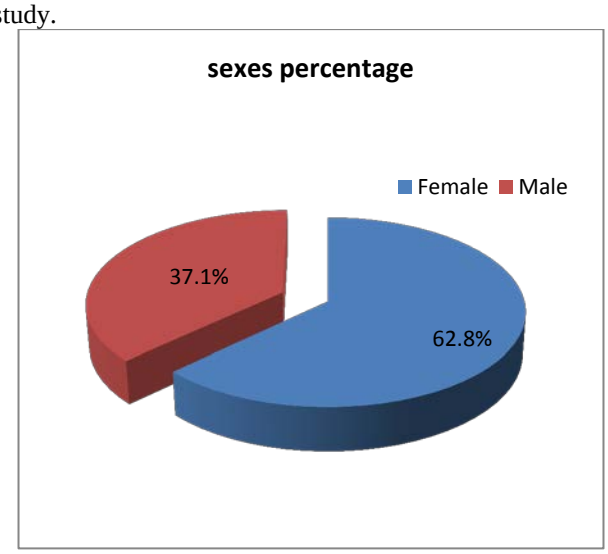

Figure (1): the percentage of sex patients in UTI samples.

Prevalence of *E.coli* Resistance against trimethoprim-sulfamethoxazole and Fluoroquinolones

The antimicrobial susceptibility test was performed for all isolates using disc diffusion test against two antibiotic belong to each Fluoroquinolones (ciprofloxacin) and Sulfonamides drugs (trimethoprim-sulfamethoxazole) The results (listed in table 2) were interpreted according to the recommendation of CLSI [32].

Table (2): The number of isolates and The result of antibiotic susceptibility test with sex of patients :

Samples	Sex	ciprofloxacin	Co-trimethoprim
1	F	S	S
2	M	S	S
3	F	S	R
4	F	S	S
5	M	R	R
6	F	S	S
7	M	R	R
8	M	S	S
9	F	S	S
10	F	R	S
11	F	R	S
12	M	S	S
13	F	S	S
14	M	S	S
15	F	S	S
16	M	R	S
17	M	R	S
18	F	S	S
19	M	S	S
20	M	S	R
21	F	S	S
22	F	S	S
23	F	S	S
24	M	S	S
25	F	S	S
26	F	S	S
27	M	R	S
28	F	S	S
29	F	S	S
30	F	R	S
31	M	R	S
32	F	S	S
33	F	S	S
34	F	S	S
35	F	R	R

F: Female , M: Male , R:Resistance, S:Sensitive

In general trimethoprim-sulfamethoxazole (Co-Tri) was the most effective one since the percentage of resistance was 14.2% while 28.5% of the isolates showed their resistance to ciprofloxacin Figure (2) shows a comparison between the percentage and the rang of resistance for all *E.coli* isolates in this study.

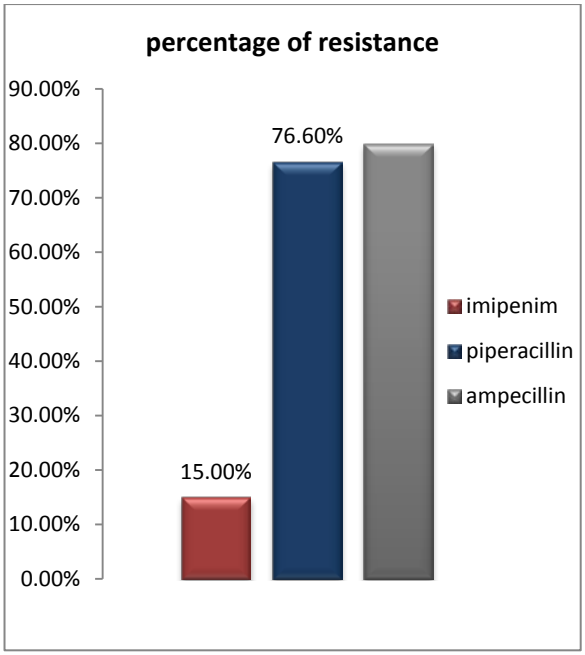

Figure (2): the percentage rate of resistance to two antibiotic against *E.coli* isolates.

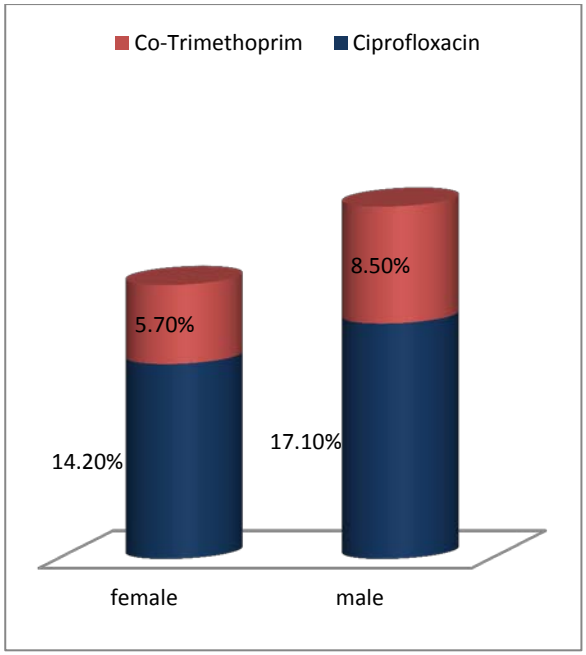

Figure (3): prevalence of resistance in sex of patients.

To discuss the results of antibiotic susceptibility for this bacteria, it was appeared that, the resistance rates of *E.coli* isolates were 14.2% (5/35) for Co-Tri thus the best activity goes to this bacteria, while it increased to 28.5% (10/35) for Cip which represent the highest resistance rate in this isolates as shown in figure (2).

In the research done by Jain *et al*,.[38] which illustrated the rate of resistance for Co-Tri antibiotic were reached to 81.85% but the ratio reached to 86.48% for Cip and these result are much highest than the percentage of this study. On the other hand in 2011, Shalini et al., [39] found that the rate of resistant to Cip is 30.44% which agree with current study. In a research done by Niranjan & Malini [40], the rate reached to (64.2% - 75%) for each Co-Tri and Cip respectively, which is also not agreement with the current study. In this study to comparison of the resistance rates according to the sex of the patients showed significant differences for resistance to Cip in male 17.1% (6/35) compared with 14.2% (5/35) in female and for Co-tri in female 5.7% (2/35) compared with 8.5% (3/35) in male as showed in figure (3).

This result is relatively close with study carried out by Alo´s *et al*,. [7] they found 25% in males and 9% in females resistance for fluoroquinolones but, not acceptable for Co-tri, who detect resistance rates for Co-tri in male 8.5% (3/35) and in female 5.7% (2/35). Three isolates $UTI_{5,7}$ of male and UTI_{35} of female were resistance to two Cip and Co-tri. Based on the data from the present study, Co-tri is currently a valid option for empirical therapy of UTI, but careful use is recommended to avoid the selection and spread of resistant strains or can use others types of drugs like Imipenem and others.

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