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IN-VITRO RESISTANCE PATTERN OF ESCHERICHIA COLI ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTION IN TEHRAN

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ABSTRACT

We aimed to nominate the sample of active bacteria resistance in Urinary Tract in Tehran as capital of Iran to find the best treatment for empirical therapy of Escherichia coli (E.coli) associated UTI for the first time in Tehran. In a descriptive cross sectional study, we studied 372 patients; 64 males and 308 females who were diagnosed with UTI. We collected all of the urine samples of patients with UTI in period of a year. First, all of the samples were collected using mid-stream clean catch method. Then, urine culture was done with 0.2 ml of urine samples in sterile containers using Eosin-methylene Blue (EMB) and blood agar special culture media (Merck, Germany). Samples were cultured in 20 minutes and plates were placed in incubator for 24hours at 37°C. All of the samples with colony count higher than 10⁵ were considered positive, then genus level (families, orders, etc.) were determined using standard methods. After culture, we transmitted antibiogram disks. After 24 hours we measured the growth inhibition zone of disk. Statistical analysis was performed using SPSS software package for windows version 15. P value less than 0.05 was statistically considered significant in all steps. We studied 372 patients; 64 males (17.2%) and 308 females (82.8%) who were diagnosed with UTI. Mean age was 44.5 years of old (1-87 y/o). In view of incidence of dysuria, frequency, urgency, hematuria, pain in flanks and pain in hypogastria. There was not a significant statistical difference in both sexes. In pregnant cases the highest susceptibility is against ciprofloxacin and the highest resistance is against Cotrimaxazole. Ciprofloxacin is first choice of antimicrobial treatment for out-patients and Cotrimaxazole and Cefexime is not supposed to be first choice of treatment.

Keywords: Urinary Tract Infection, Bacteria, In-vitro, Antibiotic Resistance

INTRODUCTION

Urinary tract infections (UTIs) having as etiologic agent Escherichia coli (E.coli) are common infections with an estimated annual global incidence of at least 250 million cases, being costly to both patients and health care funding system (Ronald, *et al.*, 2001). Non-complicated infections, particularly in women, account for the highest number of UTIs. Women diagnosed for acute uncomplicated cystitis are usually treated as outpatients: the microbiologic characteristics of this infection are highly predictable even in otherwise healthy subjects. Physicians have therefore been advised that empirical antimicrobial treatment not requiring culture is appropriate in such cases (Hooton and Stamm, 1997) Therefore, the empirical therapy has been so widely used that a few UTIs were routinely cultured. However studies clearly

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demonstrated an increasing antibiotic resistance in *E. coli* causing both community- and nosocomially acquired UTIs (Gales, *et al.*, 2000, Gupta, *et al.*, 1999).

On the basis of guidelines of Infectious Disease Society of America (IDSA) in 1999 and same association in 2011 for empirical therapy of UTI, increase in resistance of pathogens against Cotrimaxazole is reported (Warren, 1999, Gupta, *et al.*, 2011). This guideline suggests Cotrimaxazole as first line treatment for UTI if resistance is lower than 20% but current guideline informs if there is resistance around 20% (Sanford, *et al.*, 2011, Grabe, *et al.*, 2010).

In communities with higher than 20% resistance against *E.coli*, some guidelines suggest treatment with ciprofloxacin, Nitrofurantoin or Fosfomycin (Hooper and Strahilevitz, 2010, Miller and Tang, 2011). Kallen *et al.*, (2006) reported that increase in treatment with quinolones cause resistance against these antibiotics. Updated knowledge of causal bacteria and their susceptibility patterns are important for proper selection and use of antibiotics to find the best treatment for special infections there should be continuous supervision on use of antibiotics (Ronald, 2002).

It should be considered that continuous treatment with antibiotic of *E.coli* caused infection can increase the rate of resistance against antibiotics, so antibiotics should be prescribed carefully (Hillier, *et al.*, 2007). Since antibiotic treatment for Urinary Tract Infection should be based on an incident epidemiology and uro-pathological resistance pattern, this study aims to nominate the sample of active bacteria resistance in Urinary Tract in Tehran as capital of Iran to find the best treatment for empirical therapy of *E.coli* associated UTI for the first time in Tehran.

MATERIALS AND METHODS

In a descriptive cross sectional study, we studied 372 patients; 64 males and 308 females who were diagnosed with UTI. In this study that was aimed to evaluate the in-vitro pattern of resistance of *E.coli* isolated from patients with UTI in Tehran during 2012. We used convenience sampling and to ignore sampling bias we collected at least 313 samples for rejecting H0 theory. We used Chi square and Fisher exact test for evaluating H0 theory.

We collected all of the urine samples of patients with UTI in period of a year. First, all of the samples were collected using mid-stream clean catch method. Then, urine culture was done with 0.2 ml of urine samples in sterile containers using Eosin-methylene Blue (EMB) and blood agar special culture media (Merck, Germany). Samples were cultured in 20 minutes and plates were placed in incubator for 24hours at 37°C. All of the samples with colony count higher than 10^5 were considered positive, then genus level (families, orders, etc.) were determined using standard methods. To confirm the suspicious colonies of *E.coli* we used api20E kits individually for each sample. In those we needed reagent, used these test:

TDA Test: Add 1 drop of TDA reagent. A reddish brown color indicates a positive reaction.

IND test: add 1 drop of JAMES reagent. A pink color developed in the whole cupule indicates a positive reaction.

VP Test: add 1 drop each of VP 1 and VP 2 reagents. Wait at least 10 minutes. A pink or red color indicates a positive reaction. If a slightly pink color appears after 10 minutes, the reaction should be considered negative.

Finally, using these tests and on the basis of numeric profile of api 20E, determination of genus of bacteria became possible.

In samples with definite diagnosis of *E.coli*, we tried antibiogram test using Bauer-Kirby disks on Mueller Hinton agar media (PH: 7.2-7.4). Then, we passaged the bacteria on Mueller Hinton agar media and cultured carefully.

After culture, we transmitted antibiogram disks; Imipenem 10µg, Ciprofloxacin 5µg, Gentamicin 25µg, Cotrimaxazole 25µg, Cefexime 5µg, Tazocin 100+10µg and ceftriaxone 30µg which were produced in Mast company of Germany and were kept in suitable condition. Disks were settled with distance of 12mm as circle. After settling the disks, plates were kept in incubator for 24hours at 37°C.

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After 24 hours we measured the growth inhibition zone of disk using National Committee of Clinical Laboratory Standards (NCCLS) according to the manufacturer's instructions of disks and reported the results with Susceptible, relative resistance and complete resistance

In this study sensitivity of E.coli was determined by diffusion disk assay and all demographic information was collected by a questionnaire.

Statistical analysis was performed using SPSS software package for windows version 15 (SPSS Inc., Chicago, USA). Chi-Square and Fisher's exact tests were applied for categorical variables. P value less than 0.05 was statistically considered significant in all steps.

The study protocol was approved by the Ethics Committee of Tehran University of Medical Sciences, which was in compliance with Helsinki Declaration. All participants have signed a written consent which was kept completely secret.

RESULTS

We studied 372 patients; 64 males (17.2%) and 308 females (82.8%) who were diagnosed with UTI. Mean age was 44.5 years of old (y/o) with 22 years standard deviation (1-87 y/o). 194 patients (52.2%) were married while 24 (6.5%) were pregnant.

In July and February we had maximum incidence of infection (56 cases (15%)). 124 patients (32.8%) had history of previous UTI in their background while 34 had positive familial history of UTI in which they were in common with UTI in 6(1.6%), 20(5.4%), 2(0.5%) and 6 (1.6%) cases with father, mother, brother and their sister, respectively. 146 (39.2%) had history of oral agent therapy in which 140 cases were prescribed with antibiotics, 4 with immunosuppressive drugs and 2 with oral contraceptive pill. 60 (16.1%) patients had body temperature higher than 38.5 °C and 42 (11.3%) had chill at arrival.

According to the results in view of incidence of dysuria there was not a significant statistical difference in both sexes. (P=0.342), also there was not significant statistical difference in view of frequency (P=0.232), urgency(P=0.356), hematuria (P=0.179), pain in flanks (P=0.400) and pain in hypogastria (P=0.540) in both sexes. As underlying disease, diabetes mellitus (DM) was in common with 59% of the cases while nephrolithiasis and renal failure were in common with 7.7% of the patients.

Table 1: Antibiotic susceptibility pattern of E.coli in cases with UTI

Antibiotic	Susceptible	relative resistance	Complete resistance
Tazocin	186(51%)	84(23%)	95(26%)
Imipenem	181(49%)	81(22%)	17(29%)
Gentamicin	248(78%)	-	72(22%)
Cotrimaxazole	174(47%)	6(1.6%)	188(51%)
Ciprofloxacin	252(68%)	4(1%)	112(30%)
Cefexime	180(57.5%)	10(3%)	122(39%)
Ceftriaxone	214(67%)	8(2.5%)	96(30%)

In pregnant cases pattern of susceptibility is same with information in table2. As it is showed, the highest susceptibility is against ciprofloxacin and the highest resistance is against Cotrimaxazole.

Table 2: Antibiotic susceptibility pattern of E.coli in pregnant cases with UTI

Antibiotic	Susceptible	Complete resistance
Tazocin	14(70%)	6(30%)
Imipenem	15(75%)	5(25%)
Gentamicin	14(77%)	4(23%)
Cotrimaxazole	14(58%)	10(42%)
Ciprofloxacin	14(77%)	4(23%)
Cefexime	22(91.6%)	2(8.4%)
Ceftriaxone	14(77%)	4(23%)

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DISCUSSION

UTI is the most common bacterial infection world widely with prevalence of 150 million persons in a year. It is more common in females. E.coli is cause of UTI in more than the 80% of the cases.

In this study that was aimed to evaluate the in-vitro pattern of resistance of E.coli isolated from patients with UTI in Tehran during 2012, 372 patients were included in study in which 64 were male (17.2%) and 308 were female (82.8%).

Mean age was 44.5 years of old (y/o) with 22 years standard deviation (1-87 y/o). 194 patients (52.2%) were married while 24 (6.5%) were pregnant.

E.coli is the most common pathogen of UTI which causes 80% of the cases. In a study by Madani *et al.*, on 10492 cases in Kermanshah city of Iran, E.coli was cause of UTI in 45.4% of the isolates while Akram *et al.*, reported that E.coli was the cause of 60% of the cases on 920 patients in India (Madani, *et al.*, 2008–Akram, *et al.*, 2007). Also, Rostamzadeh *et al.*, showed 78.5% prevalence in 803 UTI isolates in Urmia city of Iran.

Our findings showed that prevalence of Ecoli is higher in females in comparison with males that can be due to shortness of urethra. Our findings confirm the findings of Savadkouhi and Vaez-Zadeh and their coworkers which showed that UTI is more common in females. In study by Rajab-nia in Zahedan, the highest and lowest rates of resistance were showed against Cotrimaxazole and Gentamicin while our findings showed these rates against Cotrimaxazole (51.8%) and Cefexime (39%), respectively. In our study, the highest rate of susceptibility was showed against Gentamicin (78%) and Ciprofloxacin (68%). In pregnant cases, the highest rate of susceptibility was showed against ciprofloxacin (91.6%) while the highest rate of resistance was showed against Cotrimaxazole (42%).

In a study by Shirazi *et al.*, in Hamedan, 377 pregnant women were studied to find out the prevalence of asymptomatic bacteruria in pregnancy. 10.1% of the cases had asymptomatic bacteruria. Isolated microorganisms were Escherichia coli (68.4%), Staphylococcus epidermidis (10.5%), Klebsiella pneumonia (7.9%), Entrococusfekalis (5.2%), Enterobacter cloacae, Morganellamorganii and Staphylococcus aureus (2.6%) (Shirazi *et al.*, 2008). In antibiogram test consist of 15 antibiotic disks, Ampicillin had the highest and ceftriaxone and ciprofloxacin had the lowest rate of resistance.

In a study by Safdari *et al.*, Ampicillin had the highest and Amikacin had the lowest rate of resistance which were same with the findings of Vaezzedeh *et al.*, (Safdari, Ghazvini, 2008). In a study by Madani on 10492 cases, Ampicillin had the highest and ciprofloxacin had the lowest rate of resistance (Monadi, *et al.*, 2008).

Considering these similarities in susceptibility pattern of Ecoli, Ciprofloxacin and Amikacin can be used for empirical therapy of UTI as first line of treatment. Pattern of resistance is different in different regions which changes and increases against newer antibiotics, so it is suggested to recheck and evaluate the pattern of resistance pattern every few years in different regions to find the best treatment for empirical therapy in UTI.

Conclusion

In our findings E.coli is the most common microorganism in urinary tract infection. The highest rate of resistance in invitro was showed against Cotrimaxazole (51.8%) and Cefexime (39%) in both sexes, although the highest rate of sensitivity was showed against Gentamicin (78%) and Ciprofloxacin (68%). In pregnant cases the highest rate of resistance and sensitivity were showed against Cotrimaxazole and Ciprofloxacin respectively.

On the basis of the results, Ciprofloxacin is first choice of antimicrobial treatment for out-patients and Cotrimaxazole and Cefexime is not supposed to be first choice of treatment.

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