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Prevalence of *Escherichia coli* in Suspected Urinary Tract Infected Patients and Their Sensitivity Pattern Against Various Antibiotics in Gilgit-Baltistan, Pakistan

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Abstract.- Urinary tract infection (UTI) is one of the common infections in Asian communities. Distribution and susceptibility of UTI causing pathogens change according to time and place. This study was carried out to assess the prevalence and antibiotic resistance patterns of *Escherichia coli* against various antibiotics at District Headquarter (DHQ) Hospital, Gilgit, amongst 300 suspected UTI patients referred by physicians from July to December 2012. The midstream urine samples were analyzed using a semi-quantitative culture method and uropathogens were identified by WHO guidelines. Out of 300 investigated samples, *E. coli* was isolated in 143 (47.7%) samples, while 28 (9.4%) samples showed no growth, 26 (8.7%) samples showed mixed growth, 32 (10.7%) samples were *Klebsiella pneumonia*, 41 (13.7%) were *Enterococci* spp. and 30 (10 %) were coagulase negative *Staphylococci*. In gender wise distribution more females (60%) were referred and infestation of *E. coli* was also higher in females (66.5%). The most infective age group was 21-30 years 20 (21.1%). Ceftriaxone was found to be the most effective antibiotic followed by Ciprofloxacin and Cotrimoxazole, while Amoxicillin was the least effective amongst our isolates.

Key words: *Escherichia coli*, antibiotic resistance, urinary tract infections.

INTRODUCTION

Urinary tract infection (UTI) is the third most common infection of humans after respiratory and gastrointestinal infections (Hossain *et al*., 2013). It has been estimated that about six million patients visit outpatient departments (OPD) and about 300,000 are treated in the wards every year for UTI worldwide (Bhat *et al*., 2011; Prakash and Saxena, 2013). The causative agents of UTI are developing resistance against antibiotics and treatment costing the global economy in excess of 6 billion dollars (Gonzalez and Schaeffer, 1999).

The prevalence of UTI has been reported in all age groups and in both sexes. However, it has been estimated that more than 60% women have UTI at least once in their life time (Foxman, 2002; Foxman *et al.*, 2000). It has also been reported that the rate of the infection is 10.57% higher in sexually active females and teenage girls than males and the most common bacteria involved are *Escherichia coli* (Kumar *et al*., 2002).

The most common risk factors are female anatomy, poor personal hygiene, pregnancy, urinary tract obstruction, long time catheterization, urethral reflex, spermicidal contraception, sexual intercourse and a history of UTIs (Manges *et al.*, 2008; Nahar *et al.*, 2010; Prakash and Saxena, 2013). *E. coli*, the most common member of the family Enterobacteriaceae is the main causative agent in more than 80% of all UTIs (Parkash and Saxena, 2012; Paryani *et al*., 2012; Nicolle, 2002).

Antibiotics are the only weapons against infections (Erb *et al*., 2007; Tanvir *et al.*, 2012) but the widespread and easy availability of antibiotics and self medication make UTI a problematic disease to treat. Global research data on UTI showed that the pathogens involved in causing UTI are continuously developing resistance against commonly used conventional drugs and to newer, more potent antimicrobial agents (Rajan and Prabavathy, 2012). This alarming situation arises due to the frequent misuse of antibiotics, inadequate doses and availability of antimicrobials (Tamberkar *et al.*, 2006; Okeke *et al.*, 2000). The antibiotic resistance makes UTI treatment more complicated

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and necessitates the careful use of antibiotics along with the formulations of a new one (Hasan *et al.*, 2007). The aim of the present study was to find the distribution of pathogenic *E. coli* causing UTI in the human population of district Gilgit and to determine antimicrobial resistance pattern using the disc diffusion method (Bauer *et al*., 1996). This study is also important for clinicians in order to facilitate the empirical therapy of patients where diagnostic facilities are lacking. Moreover, the data would also be helpful to the authorities formulate antibiotic prescription policies.

MATERIALS AND METHODS

Urine samples collection

From July to December 2012, 300 suspected UTI infected patients were referred by physicians of District Headquarter Hospital Gilgit. Their early morning midstream urine sample was collected in open mouth sterilized plastic containers and cultured within 4 h.

Sample dispensation and microbial growth

The urine samples were cultured according to the WHO Manual for Laboratory Investigations of Acute Enteric Infections (WHO Manual CDD/83.3) for isolation of pathogenic bacteria in four quadrants on Cysteine Lactose Electrolyte Deficient (CLED) agar by Standard Calibrated Technique (0.01 ml) (Thomson and Miller, 2003) and incubated at 37°C for 24 h. The incubation was extended for a further 24 h if growth of bacteria was negative. *E. coli* ATCC 25922 was used as the standard reference strain. After overnight incubation, the CLED agar plates were examined for significant growth of microorganisms (pure growth and > 20 colonies).

Isolation and identification of bacteria

Based on coloney forming units th UTI diagnosis was performed where the significant bacterial counts were between 1000-10,000 cfu/ml (Stewardson *et al.*, 2011). Whereas the identification of organisms was done by using general biochemical tests *viz.*, oxidase, catalase, coagulase, urease, kliger iron agar citrate and SIM (sulphate-indole-motility agar).

Antibiotic sensitivity test

The antibiotic sensitivity was performed for confirmed pathogenic *E. coli* by the standard Kirby Bauer's disc diffusion method (Bauer *et al*., 1996) and the results were interpreted by the Clinical and Laboratory Standards Institute (CLSI, 2012). Discs with 4 broad spectrum drugs, Ampicillin Group (Amoxicillin 30 µg), Cephalosporin Group (Cephradine 30µg, Ceftrizone 30µg, Naldixic Acid 30µg and Pipedimic Acid 30µg, and cefaclor 30µg) Quinolone Group (Ciprofloxacin 5ug) was also used to sustain the quality of the resistance test in each batch of *E. coli* ATCC 25922.

RESULTS

Table I shows the age and gender wise distribution of the suspected UTI patients referred to laboratory for bacteriological investigation. The highest number of patients referred were in the age group 21- 30 followed by 31- 40, 41-50, 11-20, 51 - 60, 61- 70, 0 – 10 and >70 age group. In all the age groups, the number of female patients was higher compared to the male patients.

Figure 1 shows the antibiotic sensitivity pattern of various antibiotics used against 143 *E. coli* isolated from suspected UTI patients. Out of 8 used antibiotics ceftriaxone 118 (82.5%) is highly sensitive followed by pipedimic acid 55 (38.5%), ciprofloxacin 41 (29.7%), co-trimaxazole 40 (28.0%), nalidixic acid 34 (23.8%), cefaclor 33 (23.1%), cephradine 27 (18.9% and amoxicillin 09 (6.3%). Ciprofloxacin was tested against 138 *E. coli* isolates and sensitivity pattern was 41 (29.7%). The isolated *E. coli* strains were highly resistant to amoxicillin 134 (93.7%), followed by cephradine 116 (81.1%), cefaclor 110 (76.9%), nalidixic acid 109 (76.2%), co-trimaxazole 103 (72.0%), ciprofloxacin 97 (70.3%), pipedimic acid 88 (61.5%) and ceftrixone 25 (17.5%).

Mean number of strains tested against antibiotics 97.75±11.44 and 44.62±11.50 were reported for resistance and sensitivity to *E. coli* isolates, respectively. The chi-square test for heterogeneity showed the chi-square value 13.07 for amoxicillin (30 µg) followed by 3.23 for cephradine (30 ug) and showed significant differences for antibiotics (p-value 0.001) at $p<0.05$. Data presented

Table I.- Distribution of Suspected UTI patients investigated by age and sex-wise distribution of patients infected with *Escherichia coli.*

Age (Years)	No. of cases investigated for infestation of *E. coli*			No. of cases infected with *E. coli*		
	Total	Male	Female	Total	Male	Female
0 – 10	12 (04.0%)	05 (04.2%)	07 (03.9%)	08 (05.6%)	03 (06.25%)	05 (05.3%)
11 – 20	44 (14.7%)	17 (14.2%)	27 (15.0%)	23 (16.1%)	07 (14.6%)	16 (16.9%)
21 – 30	77 (25.7%)	30 (25%)	46 (25.6%)	35 (24.5%)	10 (20.9%)	25 (26.3%)
31 – 40	68 (22.7%)	29 (24.2%)	40 (22.3%)	31 (21.7%)	11 (22.9%)	20 (21.1%)
41 – 50	45 (08.4%)	19 (15.9%)	26 (14.5%)	22 (15.4%)	08 (16.7%)	14 (14.7%)
51 – 60	26 (08.7%)	10 (08.4%)	15 (08.4%)	12 (08.4%)	04 (08.4%)	00 (08.4%)
61 – 70	24 (08%)	10 (08.4%)	17 (09.5%)	10 (07.0%)	04(08.4%)	06 (06.3%)
> 70	04 (01.4%)	00 (00.0%)	02 (01.1%)	02 (01.4%)	01 (02.1%)	01 (01.1%)
Total	300	120 (40%)	180 (60%)	143(47.7%)	48 (33.6%)	95 (66.5%)

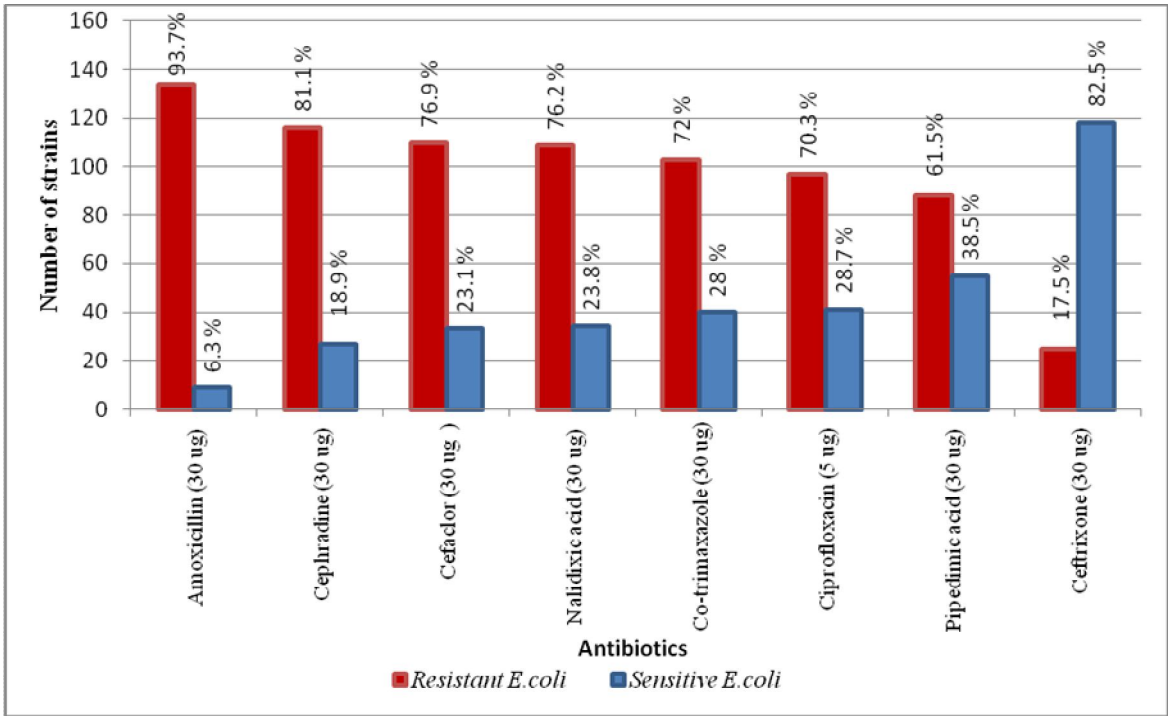

Fig. 1. Number and percentage of resistant and sensitive *Escherichia coli* strains tested for resistance and sensitivity to antibiotics for UTI infection in the patients.

in Table II revealed that it was highly significant (p value, 0.00) and most common in two age groups with values of X^2=45.60 for females and X^2= 30.40 for males in the age group of 21-30 years at $p< 0.05$.

DISCUSSION

Urinary tract infection is a medical condition which needs the proper identification of causative agents and their antibiotic sensitivity profiles is very important for the development and administration of successful local and global treatments. Gram negative bacteria have several attachment and invasion properties of the human urothelium in contrast to Gram positive pathogens. The UTI bacterial isolates in Yoon *et al*. (2011) and Akram *et al.* (2007) studies detected only Gram negative bacteria.

Table II. Chi-sqaure test for UTI pateitns (male and female) in different age groups*

Age groups	Variable		Frequency
	Female	Male	
0 – 10	7 7.20 0.01	5 4.80 0.01	12
11-20	27 26.40 0.01	17 17.60 0.02	44
21 – 30	46 45.60 0.00	30 30.40 0.01	76
31 – 40	40 41.40 0.05	29 27.60 0.07	69
41 – 50	26 27.00 0.04	19 18.00 0.06	45
51 – 60	15 15.00 0.00	10 10.00 0.00	25
61 – 70	17 16.20 0.04	10 10.80 0.06	27
> 70	2 1.20 0.53	0 0.80 0.80	2
Total Number	180	120	300

*Data in each box having three rows represents frequency, chi-square value and p value <0.05 for both male and female groups.

E. coli, the most common member of the family Enterobacteriaceae, accounts for 75-90% of all UTIs in both inpatient and out-patients (Dromigny *et al*., 2005). The presence of *E. coli* in the gastrointestinal tract, is the primary source of UTI (Sharma *et al*., 2013; Raksha *et al*., 2003). Khadka *et al*. (2012) conducted a study in Nepal and showed that out of 116 organisms, 19 were Gram negative and only 18 were Gram positive.

The results of our study show that *E. coli* is dominant (47.7%) compared to other causative agents. Similar results have been obtained in Nepal (Khadka *et al*., 2012; Sharma *et al*., 2013; Chaudhry *et al*., 2012), Kashmir (Ahmad, 2012), South India (Razak and Gurushantappa, 2012), Sindh Pakistan (Paryani *et al*., 2012) and Gilgit (Ahmed and Imran, 2008). In this study incidence is significantly higher in females (66.5%) compared to males (33.6%). Similar findings of high infestation was found in Nepal (Sharma *et al*. (2013), Chaudhry *et al*. (2012) and Khadka *et al*. (2012), Kashir (Ahmad, 2012), South India (Razak and Gurushantappa, 2012). The reason for the high incidence and therefore, the risk of UTI in females is due to their urinary tract anatomy and poor personal hygiene (Prakash and Saxena, 2013).

In our study, in age wise distribution, the incidence is much more common in the age group 21-30 years as compared to other age groups. In this age group, out of 35 patients 25 (26.3%) were females and only 10 (20.9%) were males.

These results have been endorsed by Ahmad (2012) in his study conducted in Kashmir, Razak and Gurushantappa (2012) in his study in South India, and Khadka *et al*. (2012) in their studies conducted in Nepal. In this age group, females are much more sexually active; moreover, use of spermicidal contraception, sexual intercourse and pregnancy make them even more at risk.

In our study the most sensitive drug of choice for treatment of UTI patients due to *E. coli* is ceftrixone *i.e.* 82.5% (118/143) and most resistant drug is amoxicillin 93.7 (134/143), ciprofloxacin is 70.3% (97/138) and nalidixic acid 76.2% (143/109) and co-trimaxole 72% (103/143). The *E. coli* isolates from UTI patients for the above, antibiotics are more resistant than the strains isolated by Ahmad *et al*. (2012) in Kashmiri patients and Sharma *et al*. (2013) and Khadka *et al*. (2012) in Nepal.This high pattern of resistance is due to self medication and improper use. In our area, women are shy and reserved and tend to self medicate at home. Additionally, they stop the antibiotic treatment when they feel relief from pain without completing the full dose.

Proper treatment and bacterial strains isolated from UTI patients are highly resistant to commonly used antibiotics. Ahmed and Imran (2008) also found *E. coli* strains with high antibiotic resistance in their studies.

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