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General Medicine

Drug Resistance in Uropathogenic *Escherichia coli* among Patients with Urinary Tract Infection in a Tertiary Care Hospital

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Abstract: The increasing prevalence of antibiotic resistance is a major health problem worldwide therefore World Health Organisation (WHO) has recognized the importance of studying the emergence and, the determinants of resistance and the need for strategies for its control. UTI are a common cause of morbidity in women. The majority of cases involve only the lower urinary tract and the most common pathogen is *Ecoli.* To study drug resistance in uropathogenic Ecoli among patients of urinary tract infections (UTI) in a tertiary care hospital. Mid-stream urine samples from patients clinically suspected to have UTI attending our hospital was collected after proper instructions. Antibiotic sensitivity will be done using modified Kirby-Bauer disk diffusion method. There were 65 females and 35 males with mean age of 49.79yrs. 54% had a colony count of 10^5cfu/ml; 10^4cfu/ml in 24% of the cases and in 22% of the samples, the colony count was 10^3cfu/ml. high degree of sensitivity was shown to amikacin (77%) and low sensitivity to ampicillin (20%). Among the the 100 uropathogenic *Ecoli* samples isolated in our study maximum of them (60%) were ESBL producers which owes for the resistance to various antibiotics. Most of the patients had diabetes mellitus as risk factor for UTI (84%). Urinary tract infection is an important problem in the community. Most of these infections are been landing on in a uncomplicated infection because of rising prevalence of antibiotic resistance by the organism causing it. Many community based survelliance studies are required to determine the specific risk factors associated with acquisition and to establish preventive measures within the community.
Keywords: antibiotic resistance, *Escherichia coli*, urinary tract infections (UTI).

INTRODUCTION

The increasing prevalence of antibiotic resistance is a major health problem worldwide therefore World Health Organisation (WHO) has recognized the importance of studying the emergence and, the determinants of resistance and the need for strategies for its control. Urinary tract infections (UTI) is a common community acquired bacterial disease commonly affects female outpatients. *Escherchia coli* (*E.coli)* the most common member of the familyEnterobacteriaceae, accounts for 75-90% of all UTI's in both inpatients and outpatients [1]. Uropathogenic *E.coli* causes about 90% of UTI in anatomically normal unobstructed urinary tract [2]. Other infectious diseases like wound infections, bacteraemia, meningitis, & other soft tissue infections are caused by *E.coli* [3]. The virulence of individual strains in a infection is determined by the presence and actual expression of the virulence genes present in them [2].

E.coli can exhibit multidrug resistance [4]. The prevalence of antimicrobial resistance among human clinical isolates of *E.coli* has increased in recent years. Emerging resistance limits the use of agents such as trimethoprim-sulphamethoxazole resulting in increased reliance on newer broad spectrum agents such as fluoroquinolones & extended spectrum cephalosporins. But resistance to these higher cephalosporins is also being highly encountered in hospitals [5]. This is due to production of enzyme Extended Spectrum Beta Lactamases (ESBL) among clinical isolates of *E.coli* [6]. This ESBL phenomenon began in western Europe most likely because expanded spectrum beta –lactam antibiotics were first used there clinically. Unfortunately emerging resistance now threatens the whole world [7]. The purpose of this study is to investigate the drug resistance in uropathogenic *E.coli* strains isolated from the urine cultures of patients attending our hospital. This knowledge of resistance pattern in a geographical area will helps to guide the appropriate and judicious antibiotic use.

Aim and Objectives

Aim

To study the drug resistance in uropathogenic*E.coli*in a tertiary carehospital.

Objectives

- To Isolate and identify *E.coli* from urine samples and look for significant bacteruria.
- To study the antibiotic sensitivity pattern of uropathogenic *E.coli*.
- To detect multidrug resistance in *E.coli* including the production of Extended Spectrum Beta lactamase (ESBL).
- To study the risk factor for UTI among the community.

MATERIALS AND METHODS

Study Setting and design

This is a health care based prospective study and was conducted in SDM medical college, Dharwad.

Sample size

Urine samples of 100 patients fulfilling the inclusion criteria attending our hospital was collected (with 95% confidence level and 90% power).

Mid-stream urine samples from patients suspected to have UTI attending our hospital was collected after proper instructions from 1st march 2017 to 31stMarch 2018.

Inclusion criteria

Members satisfying the signs and symptoms of UTI like dysuria, frequency, burning micturition are included.

Exclusion criteria

- Patients with indwelling catheters
- Patients with any kidney or urethral diseases
- Patients having any urethral obstructions
- Ethical clearance is obtained from the time bound research ethics committee.

Procedure

Mid-stream urine samples were collected in sterile containers and transported to the laboratory immediately and processed within two hours. Wet mount of centrifuged deposit observed for cellular exudates.5-10 pus cells per high power field was considered as pyuria. Smears were prepared, stained by gram's method and examined for the presence of pus cells and bacteria. Urine culture was done by semiquantitative method using calibrated loop (internal diameter 4mm) which delivers 0.01ml of urine .Samples were inoculated on Mac Conkey's agar and blood agar plate and is incubated at 37^0 c for 24 hours . Plates were examined for type of growth and colony count was determined. Bacteria grown in significant count (>100000CFu/ml) were identified by colony morphology, gram reaction, morphology, motility and standard biochemical reactions [8].

Antibiotic sensitivity was done using modified Kirby-Bauer disk diffusion method [8]. According to CLSI guidelines, Isolates showing inhibition zone size of less than 27 mm identified as ESBL producer and tested for ESBL production by phenotypic confirmatory test with combination disk for ESBL [9]. While performing antibiotic testing, ceftazidime (30microgram) and ceftazidime plus clavulanic acid (30/10microgram) was placed on Mueller-Hinton agar at a distance of 25mm and incubated. Organism was considered as ESBL producer if there is a >5mm increase in zone diameter around ceftazidime/clavulanate disc than that of ceftazidime disc alone [9]. *Escherichia Coli* ATCC 25922 and *Klebsiella pneumoniae*-strain 700603 were used as negative and positive controlsrespectively.

Statistical analysis

The data was collected and entered into the Microsoft excel. Sensitivity and Resistance were estimated with proportions. Appropriate charts and diagrams were shown.

RESULTS

Table-1: Prevalence of *Ecoli* according to sex and age

sex	Frequency	Percent
F	65	65.0
M	35	35.0
Total	100	100.0

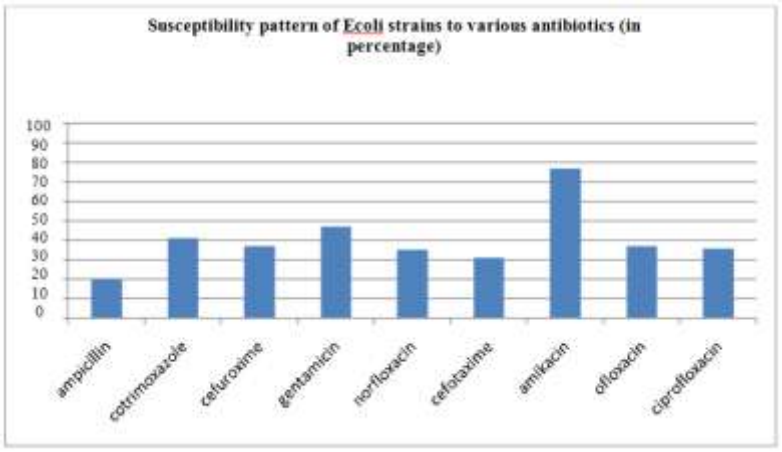
Of the 100 urine samples collected from the patients in the study, 65(65%) were females, 35(35%) were males with mean age of 49.79 yrs and std. deviation of 22.39. The male: female ratio is 1:1.85.

Minimum age was 1year and maximum age 88 years with most of them in the age group 50 to 60yrs. The study shows that UTI is more common among females.

Table-2: Frequency of colony count

Colony count(cfu/ml)	Frequency	Percent
$>10^3$	22	22.0
$>10^4$	24	24.0
$>10^5$	54	54.0
Total	100	100.0

Colony count of *Ecoli* strains in 22(22%) of them were $>10^3$cfu/ml; 24(24%) of them had the colony count of $>10^4$cfu/ml followed by $>10^5$cfu/ml in 54(54%) of the samples. About 84% of the patients had diabetes mellitus as a risk factor for UTI.

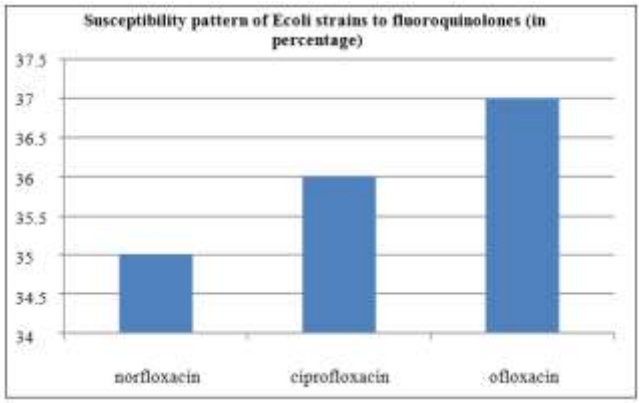


Graph-1: Antibiotic susceptibility pattern of uropathogenic *Escherichia coli* to various antibiotics (N=100): percentage)

It was found that 79(79%) of the *Ecoli* strains were resistant to Ampicillin, 20(20%) of them sensitive and 1(1%) in an intermediate range for this drug. It is noted that high susceptibility(77%) was to amikacin and low sensitivity(20%) to ampicillin.Of the 100 uropathogenic *Ecoli* strains included in our study, 59(59%) were resistant and remaining 41(41%) of them were sensitive to co-trimoxazole.

Of the 100 strains, 37(37%) were sensitive to cefuroxime whereas 67(67%) of them are resistant to the drug. 47(47%) of the strains were sensitive to gentamicin (aminoglycoside); 49(49%) were resistant followed by 4(4%) in the intermediate range. Only 35(35%) *Ecoli* strains were sensitive to norfloxacin; 2(2%) are in intermediate range with 63(63%) strains showing resistance to the drug.

Cefotaxime was found to be sensitive only in 31(31%) cases whereas in remaining 69(69%) cases it was seen resistant.Of the 100 samples included in our study, 77(77%) strains showed sensitive to amikacin whereas 13(13%) were resistant and 10(10%) of them are in the intermediate range 63(63%) strains showed resistant to ofloxacin and remaining 37(37%) were sensitive to this drug. Ciprofloxacin (fluoroquinolones) was sensitive to about only 36(36%) *Ecoli* strains and was resistant to 64(64%) strains respectively



Graph-2: Fluoroquinolone susceptibility of uropathogenic *Ecoli* strains (N=100) Graph 3:

Among the fluoroquinolones, 35%sensitivity to norfloxacin, 36% susceptibility seen to ciprofloxacin followed by 37% sensitivity to ofloxacin.

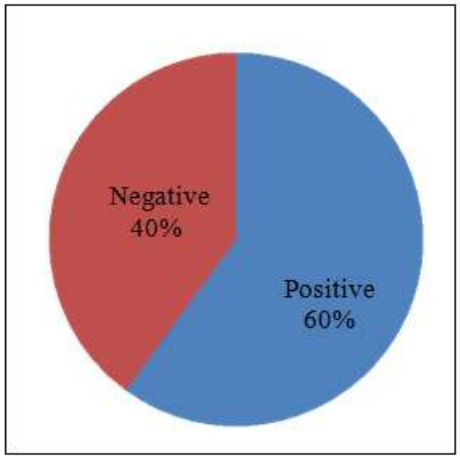


Fig-1: Pattern of ESBL production by uropathogenic*Ecoli*:

Out of the 100 uropathogenic *Ecoli* strains isolated from patients with UTI, 60(60%) were ESBL producers whereas remaining 40(40%) were non-ESBL producers which is evident from our study.

Also, diabetes mellitus was seen as major risk factor among 84% of patients included in the study.hence adequate control of blood glucose is utmost necessary.

DISCUSSION

The antimicrobial resistance of bacteria is a problem of global concern. There is a correlation between antibiotic use and subsequent resistance. Antibacterial consumption is increasing in many countries around the world, and is recognised as main reason for the emergence of resistance.

In our study, out of the 100 *Ecoli* strains isolated from the patients with UTI, 65% of them were females and 35% were males with male to female ratio is 1:1.85. This is in contrast to the study done by David C Bean *et al*., [12]. This is mainly because the female urethra appears to be particularly prone to colonization with uropathogenic *Ecoli* because of its proximity to the anus, its short length (~4cm), and termination beneath the labia. Maximum age of the patient included in our study was 88yrs and minimum age was 1yrs with the mean age group of 49.79yrs having standard deviation of 22.39. Most of the patients were in 50-60 yrs.

About the frequency of the colony count in our study, 54% of them had significant bacteriuria with colony count $>10^5$ cfu/ml. In 24% of the patients the colony count was $>10^4$ cfu/ml; followed by $>10^3$ cfu/ml in the remaining 22% of patients. About 46% of the patients having true UTI is lacking significant bacteriuria. Especially in symptomatic patients, fewer bacteria (10^2-10^4cfu/ml) will signify infection [13].

The prevalence of antimicrobial resistance among human clinical isolates of *Ecoli* has increased dramatically these recent years. In our study, *Ecoli* were studied for antibiotic susceptibility pattern which included ampicillin, co-trimoxazole, norfloxacin, ofloxacin, ciprofloxacin, amikacin, cefuroxime, gentamicin, and cefotaxime. Resistance were observed to antibiotics such as ampicillin, co-trimoxazole, cefotaxime, and gentamicin. Maximum number of isolates (79%) were resistant to ampicillin and lowest (13%) to amikacin. These results are in consistent with the previous studies on drug resistance in *Ecoli* [14]. Greater prevalence of resistance to common antibiotics have alsobeen reported by other workers [4, 12].

Co-trimoxazole susceptibility of *Ecoli* strains was 41%. In a study done by David C Bean *et al*., co-trimoxazole susceptibilities was 40% [12] where it was 43.5% in the study done by M. J. Saffar *et al*., A bit large difference is seen in the susceptibilities to co-trimoxazole by us compared to studies done elsewhere. In our study, only 41% *Ecoli* strains were sensitive to cotrimoxazole while various other studies have noted susceptibilities ranging between 90%-99% [14]. The ampicillin and trimethoprim-sulphamethoxazole resistant Ecoli implies that another antibiotic should be used for empirical treatment and that there is a need for generic drugs in developing countries.

Norfloxacin, Ciprofloxacin and ofloxacin, were used as a representative of the fluoroquinolone group of antibiotics in the present study. Sensitivity of *Ecoli* strains to ciprofloxacin, ofloxacin and norfloxacin are 36%, 37% and 35% respectively which is evident from the study. Comparatively a high degree of resistance is noted to ciprofloxacin (64%) when compared to other two drugs.

Increasing resistance among these drugs has been reported in other studies also [15]. The increased use of fluoroquinolones for the empirical therapy of UTI may be responsible for promoting resistance to these drugs.

Gentamicin susceptibility of the *Ecoli* strains were 47% in our study whereas it was 65% in the other study [16]. Amikacin susceptibility of the organism is the maximum (77%) that is evident from our study. These are in in close proximity with other studies, where it is 92% in the study done by Yasmeen Kausar *et al*., and 93.5% in the study done by M. J. Saffar *et al*., [13]. In our study, out of 100 *Ecoli* strains 31% were susceptible to cefotaxime which is comparable to 40.5% susceptibility in the study done by Yasmeen Kausar *et al*., [17].And cefuroxime susceptibility in our study was found to be 41% with 59% of them being resistant to the drug whereas 30% sensitivity is seen in the study done by Kalem *et al*., [10] which is in consistent with our study. In the another study done by Hande Arslan *et al*., only 23% sensitivity to cefuroxime was observed. We observed that, a high rate of (60%) ESBL production of *Ecoli* which may be due to selective pressure imposed by extensive use of antimicrobials. The indiscriminate use of cephalosporins is responsible for high rate of selection of ESBL producing organisms. The results are in consistent with previous from India [11]. National survey have indicated the presence of ESBLs in 5% to 8% of Ecoli isolates from Korea, Japan, Malaysia, and Singapore but 12% to 24% in Thailand, Taiwan and the Phillipines and Indonesia [18]. However, there are differences from hospital to hospital. Use of a variety of other antibiotic classes has been found to be associated with subsequent infection due to ESBL producing organisms. These include quinolones, co-trimoxazole, aminoglycosides [19]. In the study done by Jean Baldus Patel, the ESBL production by uropathogenic *Ecoli* was found to be 51.5% which is consistent with our study.

CONCLUSIONS

In the view of the emerging drug resistance amongst bacteria, therapy should only be advocated, as far as possible, after culture and sensitivity has been performed. This would not only help in proper treatment of the patients but would also discourage the indiscriminate use of antibiotics and prevent further development of bacterial resistance with adequate glycaemic control.

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