ORIGINAL ARTICLE

Prevalence and Fluoroquinolone Resistance Pattern in *Escherichia coli* Isolates of Urinary Tract Infection (UTI) Patients

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Abstract:

Background: Urinary tract infections (UTIs) are among the most common infectious diseases all over the world. Recent studies reported an increased antibiotic resistance in *Escherichia coli*, primary causative agent of UTI. The resistance has emerged even to more potent antimicrobial agents like fluoroquinolones. *Objectives:* The present study was undertaken to evaluate the prevalence and resistance pattern of *E.coli* causing UTIs in patients admitted to a tertiary care hospital in South India, with reference to fluoroquinolones. *Material and Methods*: A total of 278 selected urine samples of urinary tract infections were processed for *E.coli* culture using standard methods. For these urinary *E. coli* isolates, susceptibility to various antibiotics including fluoroquinolones was checked by Kirby Bauer disk diffusion method according CLSI criteria. Final resistance to fluoroquinolones isolates was analyzed. *Results:* Out of the 278 selected UTI clinical isolates 148 (54%) showed ciprofloxacin sensitive and 130 (46%) clinical isolates are ciprofloxacin resistant. Of the 130 ciprofloxacin resistant urinary isolates of *E. coli* subjected to susceptibility test for increased generation of fluoroquinolone drugs, the pattern of resistance noticed as levofloxacin (2nd generation) 79%, gatifloxacin (3rd generation) 77% and moxifloxacin (4th generation) 75%, respectively. The fluoroquinolone resistance in UTI clinical isolates was decreasing with increasing generations of fluoroquinolone. Quinolone drug resistance in clinical isolates was increasing with age and hospitalized patients. *Conclusion:* Study showed an increased fluoroquinolone resistance among uropathogenic *E. coli* isolates of UTI. These increased antibiotic resistance trends in UTI patients indicated that it is imperative to rationalize the use of antimicrobials and to use them conservatively.

Keywords: Urinary tract infections, *Escherichia coli*, Antibiotic sensitivity, Fluoroquinolones

Introduction:

Urinary tract infections (UTI) are among the most common infectious diseases encountered in clinical practice all over the world with a high rate of morbidity and economic burden to health care systems [1]. It is the second most common type of infection in the body. According to American Urological Association (AUA), it is estimated that 150 million people infected with UTI per year worldwide, accounting for $6 billion in health care expenditures. Urinary tract infection is a bacterial infection that affects any part of urinary tract [2]. The manifestations of

UTI may vary from mild asymptomatic cystitis to pyelonephritis and septicaemia [3].

The commonest bacterial agent involved in causation of UTIs is *Escherichia coli*, being the principal pathogen both in the community as well as in the hospital [4].

UTIs in hospital and community setting are initially treated empirically based on frequency of pathogens, local antimicrobial resistance rates and illness severity. Treatment of UTI constitutes a great portion of prescription of antibiotics. Urinary pathogens have shown a changed pattern of susceptibility to antibiotics, resulting in an increase in resistance to commonly used antibiotics. Fluoroquinolones are preferred as initial agents for empiric therapy because of high bactericidal and clinical cure rates as well as low rates of resistance among uropathogens [5]. Recently, several studies have revealed increasing trends of resistance even to fluoroquinolones [6-10].

The emergence of fluoroquinolone resistant uropathogenic *E. coli* is of great concern because these pathogens account for 20% of all hospital acquired infections [10-13]. After notifying the role of fluoroquinolones in UTIs caused by *E. coli*, the present study is undertaken to study resistance towards urinary *E. coli* with various generations of fluoroquinolones and also to assess sensitivity pattern of other drugs in place of fluoroquinolones resistant *E. coli* urinary tract infections with an objective to define appropriate intervention strategies to be applied in patient care and management.

Material and Methods:

Study site: The study was carried out in the Department of Microbiology, Narayana Medical College and Hospital, Nellore, India, during January 2009 to October 2009. The study included of 278 selected urinary samples of *E.coli* isolates with urinary tract infection from the patients who attended to Narayana General Hospital both as inpatients and outpatients of both sex in the age range between 16 to 60 years.

A detailed history of patient including age, sex, socioeconomic status, previous history of urinary tract infections, previous history of antibiotic use, any anatomic abnormalities, hospitalization etc were recorded in the prescribed proforma.

Patients who were excluded from the study were fluoroquinolone sensitive *E. coli* isolates, pregnant, lactating or premenopausal women, patients having nosocomial UTI, patients who had taken antibiotic treatment within 3 days prior to initial visit, patients having genital-urinary tract disease or abnormalities, patients having gastrointestinal symptoms, patients who were taking antacids, patients who were taking antirhythmic agents or other medications known to cause QTC prolongation or who had shown previous hypersensitivity or who were prone to photo-sensitivity to fluoroquinolones, or repeat isolates of *E. coli* isolated from urine of the same patient taken within two months were ignored.

Samples received included mid-stream clean catch urine, suprapubic aspirate, urine collected in a sterile, wide mouth, leak proof, labelled universal container. Prevention of contamination

by normal flora of vaginal, perineal and anterior urethra was the consideration for collection of a clinically relevant urine specimen. One sample per patient was collected to avoid duplication. As urine being an excellent supportive media for growth of most of the bacteria, it was processed immediately within one hour without delay. If there was any delay in processing, samples were stored at 2^0C to 4^0C until processed. Samples were processed and isolates were identified as per standard methods [14, 15]. All uncentrifuged urine samples were inoculated onto MacConkey agar and blood agar medium (Himedia, Mumbai, India) using a calibrated loop (volume-0.005 ml) and were incubated for 18-24 hours at 37˚C. Colonies that were positive for lactose and indole were presumptively identified as *E.coli*. Colony count was done to identify significant bacteriuria; colonies more than 50 in number were included in the study.

The antibiotic susceptibility testing of the isolated bacteria was carried out by the Kirby-Bauer method using Mueller-Hinton agar media in accordance with Clinical Laboratory Standards Institutional (CLSI) guidelines with control strain *E.coli* ATCC 25922 [16]. After adding inoculum, specified antibiotic discs placed 2 cm apart from each other with sterile forceps and were incubated for 16-18 hours at 37°C aerobically. The degree of sensitivity was determined by measuring zone of growth inhibition around the disc. The growth of bacterium would be inhibited around the discs containing antibiotics to which the bacterium is susceptible, while no inhibitory zone around resistant ones. The results were interpreted as sensitive, intermediately sensitive and resistant to the different drugs. The zone of inhibition was interpreted according to the Kirby-Bauer antibiotic sensitivity chart.

Antibiogram routinely used to test for *E. coli* urinary isolates i.e. cefipime, cotrimoxazole, nitrofurantoin, ciprofloxacin, amikacin, and sulbactum/cefoperazone and having ~40 antibiotics was used. The isolates that were resistant to ciprofloxacin were tested to other fluoroquinolone with single fluoroquinolone taken from each generation as a surrogate marker for increased generations: 1st generation - Ciprofloxacin, 2nd generation - Levofloxacin, 3rd generation - Gatifloxacin, and 4th generation - Moxifloxacin were included in the antibiogram.

Statistical analysis:

All data were tabulated and analyzed. Descriptive statistics were used for analysis, and the results were expressed as frequency and percentage. Microsoft Excel 2013 software was used to analyze the data.

Results:

Of the 278 selected UTI samples analysed for various antibiotics included with ciprofloxacin, 148 (54%) of isolates were ciprofloxacin sensitive and 130 samples (46%) are ciprofloxacin resistant. Of 148 ciprofloxacin sensitive isolates, 86 isolates were from female patients and 62 were from male patients (Fig. 1A).

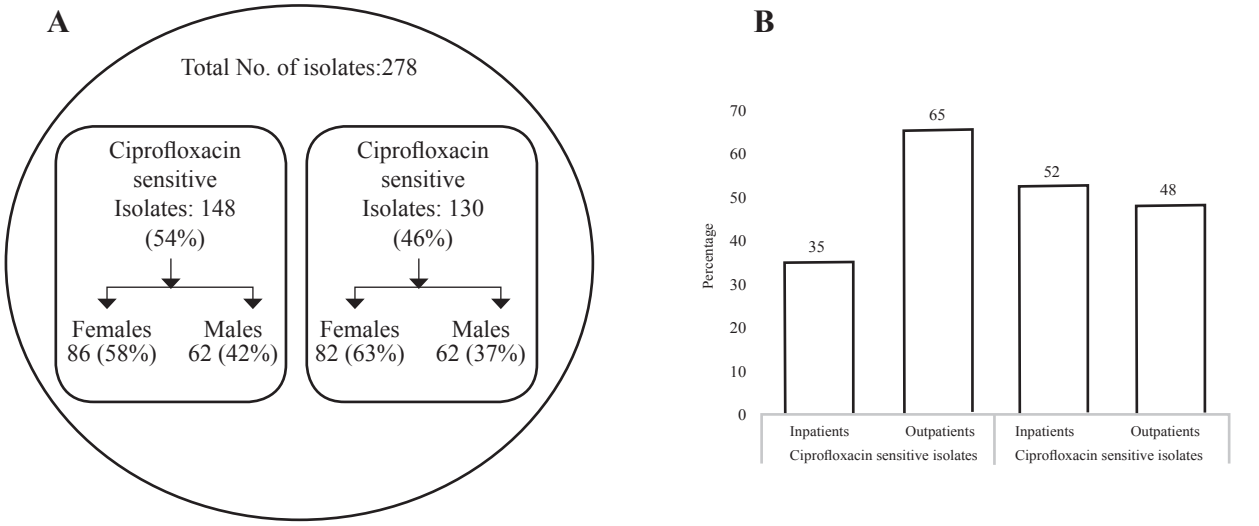

Fig. 1 : Distribution of *E.coli* isolates in Urinary Tract Infections

Among these sensitive samples, 96 were community acquired UTI samples and 52 isolates were hospital acquired UTIs. However, in the 130 ciprofloxacin resistant *E.coli* isolates 52% are inpatients of hospital and 48% were outpatients (Figure 1B). Of the 130 ciprofloxacin resistant isolates, majority of the isolates (28%) were from the age group of 56-60 years followed by 25% from age group 46-55 years, 21% from age group 36-45 years, 17% from age group 26-35 years and least (9%) in 16-25 years age group. It was also reflected that 79% of ciprofloxacin resistant isolates were identified in above 35 years of age and only 21% of infections in below 35 years of age (Fig. 2A). In the study group of 130 ciprofloxacin resistant *E.coli* isolates, 87 (67%) UTIs were seen in females than 43 (33%) in men (Table I and Fig. 2B). The resistance ratio between females to males was 2.01:1.2. Socio-economic survey in the study group revealed that the low socio-economic status showed high infection rate 62% followed by middle class 30% and higher class 8%, respectively (Fig. 2C). Of 130 ciprofloxacin resistant *E.coli* isolates, large number of samples were the referrals from Urology department (52%), followed by Obstetrics and Gynaecology (30%), Medicine (16%), and Endocrinology (2%) departments.

Table 1: Distribution of Age, Gender and Social-Economic Status among Ciprofloxacin Resistant Patients

Characteristics	Resistant samples	
	Numbers	Percentage
Age		
16 – 25	12	9%
26 – 35	22	17%
36 – 45	27	21%
46 – 55	32	25%
56 – 60	37	28%
Sex		
Male	43	33%
Female	87	67%
Socio-economic status		
Low	81	62%
Middle	39	30%
High	10	8%

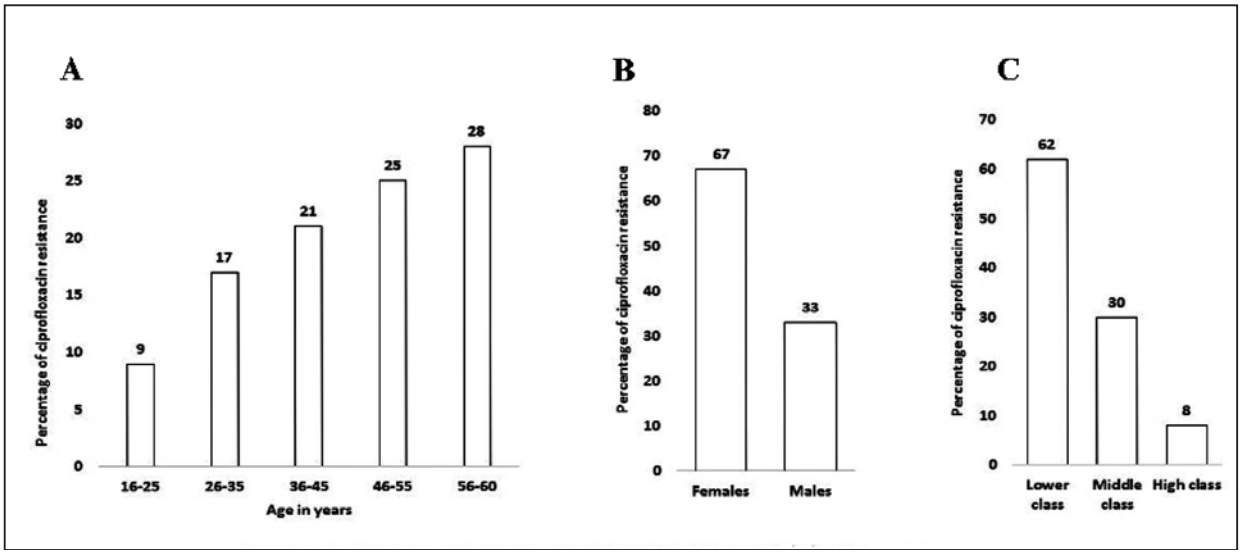

Fig. 2 : Pattern of Ciprofloxacin Resistance as a Function of Patient Age (A), Gender(B) and Social Status (C).

Further to know fluoroquinolone resistance in higher generation drugs, all 130 ciprofloxacin resistant isolates were subjected to antimicrobial susceptibility test by using 2nd, 3rd, 4th generation of fluoroquinolones under NCCLS guidelines. We noticed a slight decrease in resistance for increasing generations of fluoroquinolones (Table 2).

The 2nd generation fluoroquinolone drug levofloxacin was showing 79%, 3rd generation drug gatifloxacin 77% and 4th generation drugs

moxifloxacin showing 75% resistance with increased sensitivity from 21% levofloxacin, 23% gatifloxacin and 25% moxifloxacin, respectively. These results indicated that fluoroquinolone resistance in UTI clinical isolates was decreasing with increasing generations of fluoroquinolones. Quinolone drug resistance in clinical isolates increased with age and among hospitalized patients.

Table 2: Resistance Patterns of Increased Generations of Fluoroquinolones against Ciprofloxacin Resistance *E. Coli* Isolates of Urinary Tract Infections

Fluoroquinolone drug	Resistance	Sensitivity
Levofloxacin	79 %	21 %
Gatifloxacin	77 %	23 %
Moxifloxacin	75 %	25 %

Discussion:

Urinary tract infections are the most common bacterial infections in women and accounts for significant morbidity and increases health care costs [17]. UTI is an old problem that continues to present new challenges due to change in the aetiology of UTI and in the antimicrobial susceptibility of urinary pathogens over the years. Factors such as the changing in patient population and extensive use and abuse of antimicrobial agents could contribute to changes in the microbial profile of urinary tract isolates. *E. coli* is one of the most common bacteria capable of causing infection in humans, particularly urinary tract infection UTI [18, 19].

Although fluoroquinolones are among the most effective drugs in the treatment of UTI [20], diverse studies have reported increasing resistance to fluoroquinolones due to an increase in quinolone prescriptions. Usually, the prevalence of fluoroquinolone resistance is related to the intensity of antibiotic use [12, 21].The comparison of ciprofloxacin resistance patterns of uropathogenic *E.coli* in various studies from India and other parts of the world has shown a range from 6 % to 75% [22-27]. In our study, 46% isolates of *E.coli* were ciprofloxacin resistant. This is quite within the range. The emergence of resistance for fluoroquinolones is multifactorial [27, 28 –30]. The emergence of resistance was predicted on molecular grounds, because single mutation which raises the minimum inhibitory concentration (MIC) of ciprofloxacin by 4 to 16 folds leading to resistance.

The present study reveals the increased fluoroquinolone resistance with patient age due to decreased immune function and frequent exposure of fluoroquinolones. A higher rate of resistance is noted in hospitalized patients than out patients. Consistent with these results, Boyd *et al* [30] also have reported that fluoroquinolone

resistance has increased with time, patient age and is higher in hospitalized patients than outpatients. This may be due to decreased immune system with super added hospital acquired infections and with indwelling catheters, frequent use of fluoroquinolones and with complicated infections. They also noticed higher resistance in males than in females because of collection of large urinary isolates from male outpatients. (Fig.2). According to Spanish national surveillance study female: male *E. coli* UTI infections are 19%**:** 28.9 % [31] and our results are 67%**:**33%. However, 67%:33% is same as 2.01: 1.2

All 130 ciprofloxacin resistant *E. coli* UTI isolates further tested to antimicrobial susceptibility for 2nd, 3rd, and 4th generation of fluoroquinolones to know the sensitivity and observed increased sensitivity towards increased generation of fluoroquinolones, Levofloxacin 21%, followed by Gatifloxacin 23% and highest sensitivity 25% for Moxifloxacin (Table 2). Fluoroquinolones directly inhibit DNA synthesis by interaction of drug with DNA and other target enzymes viz. gyrase and topoisomerase IV. Other studies have also reported decreased resistance of UTI *E. coli* to fluoroquinolones [30, 32]. Fluoroquinolone resistance is typically encoded chromosomally. This resistance against fluoroquinolones in our study may reflect significant antibiotic pressure in the environment due to frequent exposure rather than co-carriage of this resistant gene on plasmids.

Fluoroquinolones resistance is higher in developing countries than in developed countries. It is supported by the socio-economic distribution in the study subjects which reveals that the lower socio-economic status shows higher rate infection 62% followed by middle 30% and higher class with 8%. Fluoroquinolones antibiotic resistance is becoming a big problem to public health which threatens the lives of hospitalized individuals as well as those with chronic conditions and adds considerably to health care cost. Therefore it is an important issue to be addressed by policy makers to formulate a strict fluoroquinolone prescription policy for urinary tract infections in our country.

In conclusion, the present study showed an increased fluoroquinolone resistance among uropathogenic *E. coli* isolates. Increased antibiotic resistance trends in UTI patients indicated that it is imperative to rationalize the use of antimicrobials and to use them conservatively.

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