



Original Research Article

A study of antibiotic sensitivity pattern and detection of fluoroquinolones resistance to *Escherichia coli* from urinary tract infections

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ABSTRACT

To know effect of Antibiotics and Fluoroquinolones resistance of *E.coli* from urinary tract infection, 100 urine samples with growth of *E.coli* were selected for this study. Clean catch midstream urine was collected from patients and subjected for Gram's stain and cultured on Nutrient agar, Blood agar & Mac Conkey's medium. *E.coli* was identified based on standard pattern of colony characters & biochemical tests. Antibiogram was done by Kirby- Bauer disc diffusion method on Muller hinton agar. Isolated organisms shown highest resistance to Cotrimoxazole (85%) followed by ciprofloxacin(63%), and Cefipime (59%). Sulbactam/Cefoperazone (70%), Nitrofurantoin (69%) and Amikacin (63%) showed very high sensitive patterns. patients were treated with higher generations of fluoroquinolones shown slight decrease in resistance rates of both decreased sensitive's and resistance rates combined as 80% for first generation drugs (Ciprofloxacin),79% for second generation drugs (Levofloxacin),77% for third generation drugs-(Gatifloxacin); 75% for fourth generation drugs(Moxifloxacin). Fluoroquinolones antibiotic resistance is becoming a big problem for 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 fluoroquinolones antibiotic prescription policy for urinary tract infections in our country.

Keywords

Urinarytract infection,
E. coli,
Fluoroquinolones

Introduction

Urinary tract infections are second most common infections in community practice. About 150 million people are diagnosed with urinary tract infections each year. *Escherichia coli* accounts for 75% to 90% of urinary tract infection isolate causing global economy in excess of 6 billion US Dollars

(Zakaria EI ASTal., 2005). Urinary tract is one of the most common sites of *Escherichia coli* infections, particularly in females. In men it is less common and occurs after 50 years of age. Urinary tract infection can be divided into two general anatomic categories i) Lower urinary tract infection (urethritis,

cystitis), ii) Upper urinary tract infection (pyelonephritis, prostatitis)

Urinary tract infections are dangerous in older people, diabetes, pregnant women, immunosuppressant, previous infections, presence of urinary catheters, and presence of decubitus ulcer when they involve kidney where the organisms cause blood poisoning (David Rahan M.D facog; 2008). Most urinary tract infections in hospital and community setting are initially treated empirically based on frequency of pathogens, local antimicrobial resistance rates and illness severity.

Fluoroquinolones are preferred as initial agents for empiric therapy because of high bacteriological & clinical cure rates as well as low rates of resistance among uropathogens. Clinical cure rates with fluoroquinolones for 4 to 6 weeks is 76.9% to 78.3% according to 1998 J. American society of microbiology (Henry D, et al; 1998) Fluoroquinolones are also recommended for patients with recurrent urinary tract infections, treatment failures and allergies to other antimicrobial agents.

However in the past few years, fluoroquinolones have been prescribed more frequently in out patients for the treatment of urinary tract infections have lead to increase in fluoroquinolones resistant *Escherichia coli* infections which is difficult to treat (Milan I cizman; et al 2001). Although antibacterial agents are universally recognized as having no antiviral activity, 50% or more patients diagnosed with a viral urinary tract infection are prescribed a course of antibacterial therapy.

Fluoroquinolones resistance for *Escherichia coli* in urinary tract infections patients being a big problem for public health which threatens the lives of hospitalized

individuals and also those with chronic infections (or) recurrent infections which can add considerably to health care cost. The study of emergence of fluoroquinolone resistant uropathogenic *Escherichia coli* is of great concern because these pathogens account for 20% of all hospital acquired infections. After notifying the role of fluoroquinolones in urinary tract infections caused by *Escherichia coli*, the present study is undertaken to study resistance towards urinary *Escherichia coli* with various generations of fluoroquinolones.

Materials and Methods

The present study included 100 urinary samples showing *Escherichia coli* isolates with urinary tract infection from the patients who attended to Narayana General Hospital both as inpatients and outpatients for a period of 6 months from Nov 2007 to May 2008. Patients who were excluded from the study were pregnant, lactating or premenopausal women, patients having nosocomial UTI, patients who had taken antibiotic treatment within 3 days prior to initial visit, patients who had genito-urinary tract disease or abnormalities that may preclude evaluation of therapeutic response. Patients who had gastrointestinal symptoms or conditions that might preclude adequate drug absorption or who were taking antacids were also excluded. Patients who had prolonged congenital electro cardio graphic QT syndrome or who were taking antiarrhythmic agents or other medications known to cause QTC prolongation or who had shown previous hypersensitivity, photo sensitivity to fluoroquinolones, or repeat isolates of *Escherichia coli* isolated from urine of the same patient taken within 2 months were ignored.

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 was recorded. This study was conducted in Narayana medical college and hospital, Nellore to compare the frequency and fluoroquinolones resistance pattern in *Escherichia coli* isolates. The study is important for clinician in order to facilitate the empiric treatment of patients and management of patients with symptoms of urinary tract infections.

Clean catch mid stream urine was collected in a sterile, wide mouth, leak proof, labeled universal container. One sample per patient was collected to avoid duplication. Grams staining was done to screen each sample to select the sample for further processing. A semi quantitative method was adopted for primary isolation of organisms using a calibrated loop with 4mm diameter, which delivers 0.01 ml of urine (Bailey and Scott's forbes; 2012). Urine samples were inoculated on Mac conkey agar and blood agar media plates and incubated at 37⁰ C over night. Colonies that were positive for lactose and indole were presumptively identified as *Escherichia coli* (Mange's AR; etal; 2001). Colony count was done for significant bacteriuria: colonies more than 50 in number were included in the present study.

On the basis of colony morphology and cultural characters, the isolates were further subjected to a series of biochemical reactions for identification. Motile gram negative bacilli with lactose fermenting colonies on Macconkey agar and hemolytic on blood agar with indole positive, methyl red test positive, triple sugar iron test negative and biochemical reactions along with sugar reactions with sucrose not fermented but A/G is seen in Glucose, Maltose, Xylose, Lactose and Mannitol were

considered for fluoroquinolones resistance in urinary *Escherichia coli* antibiogram study. Antibiotic susceptibility testing was done by Kirby – Bauer disc diffusion technique by using Mueller-Hinton agar media in accordance with the NCCLS Quality control strains *E.coli* ATCC 25922 (Mackie and McCartney). Antibiogram routinely used to test for *Escherichia coli* urinary isolates were Cefipime, Cotrimoxazole, Nitrofurantoin, Ciprofloxacin, Amikacin, and Sulbactam/Cefoperazone.

The isolates which were resistant to Ciprofloxacin were tested with single fluoroquinolone taken from each generation as a surrogate marker for increased generations. The following fluoroquinolones were used for further testing: (I Generation: Ciprofloxacin, II Generation: Levofloxacin, III Generation: Gatifloxacin, IV Generation: Moxifloxacin)

Result and Discussion

This study group of 100 *Escherichia coli* isolates of urinary tract infections showed as majority of them are in age group 56-65 (30%) followed by 28% (46-55), 21% (36-45), 12% (26-35) and least in 16-25yrs age (9%). *Escherichia coli* causing urinary tract infections were seen mostly in females 67 (67%) than in men 33 (33%). Females: Males fluoroquinolones resistance ratio is 2.03:1%. *Escherichia coli* used for study were isolated from urine samples, 52% from inpatients of hospital and from 48% were from outpatients who were suffering from urinary tract infections. The study group belonged to low socioeconomic status (62%) followed by middle (30%) and high class (8%). *Escherichia coli* used for study are isolated from the urine samples which are referrals from Urology department (52%),

followed by Obstetrics and gynaecology department (30%), Medicine department (16%), and Endocrinology department (2%).

Antimicrobial susceptibility testing to *Escherichia coli* strains isolated from urine samples showed highest resistance to cotrimoxazole (85%), followed by Ciprofloxacin (63%), cefipime (59%), Nitrofurantion (26%), Sulbactam/Cefoperazone (24%), Amikacin (20%) and highest sensitive shown to Sulbactam/Cefoperazone (70%), followed by Nitrofurantoin (69%) and in Amikacin (63%). With the use of higher generations of fluoroquinolones for treating *Escherichia coli* causing urinary tract infections, my study showed slight decrease in resistance rates of both decreased sensitive's and resistance rates combined as 80% for ciprofloxacin-first generation drugs, 79% for second generation drugs-levofloxacin, 77% for third generation drugs-gatifloxacin; 75% for fourth generation drugs-moxifloxacin. (Table 1 and Table 2)

Urinary tract infections are the most common bacterial infection in women and accounts for significant morbidity and increases health care costs (Iravani A; 1991). Fluoroquinolones resistance increased significantly with patient age because of decreased immune function and overall more frequent fluoroquinolones exposure than that for younger patients (Lauron Becnel Boyd; et al; 2008). Female: Male *Escherichia coli* urinary tract infections is 19%: 28.9% in 2002 – according to Spanish national surveillance study and my study shows 67%:33 % (Eva Moreno ; et al; 2006). Increased fluoroquinolone resistance towards urinary *Escherichia coli* in females is due to short urethra and repeated infection. Less in males is due to prostatic secretions that contain zinc which acts as bactericidal substance and

lactobacilli in females reduces the ability of microorganisms attach to uroepithelial cells and do not penetrate (THOMPSON. C; 2001).

Inpatients and outpatients resistance rates of fluoroquinolones to *Escherichia coli* strains isolated from patients with urinary tract infections rose from 11.8 to 43.3% for outpatients and from 33.9% to 46.5% for inpatients but my study showed as 52% for inpatients and 48% for out patients according to study by many authors due to decreased immune system with super added hospital acquired infections and with indwelling catheters, frequent use of fluoroquinolones and with complicated infections (Albert Sotto; 2001). When data from all fluoroquinolones tested in a particular year were combined, the frequency of resistance increased to 25% in my study when compared to 6% of Boyd et al 2008 (Lauron Becnel Boyd; et al; 2008). In china ciprofloxacin resistance is increasing 46.6 to 59.4% in 1998- 2002 studies while it is 62% and 18% with decreased sensitivity in my 2008-2009 study.⁽¹⁾ All fluoroquinolones except moxifloxacin were associated with increased sensitivity while moxifloxacin is associated with decreased sensitivity due to increased usage of moxifloxacin during 2003-05. My study is showing that all fluoroquinolones were associated with decreased sensitivity due to increased usage except moxifloxacin which showed increased sensitivity due to decrease usage after 2005 (Richard J. Rayan; 2008). Milan et al pointed out that the abusive and anarchic use of fluoroquinolone is probable the leading factor for the high percentage of resistance (Milan I cizman; et al; 2001). Increasing prescriptions of fluoroquinolones as first line therapy for common urinary tract infections has facilitated emergence of resistance by double mutations at gyrase A and par C at

positions 248 (T) & 259 (A) confirms the high fluoroquinolones resistance rates towards urinary *Escherichia coli* in my study, which showed important region for molecular basis to be studied by a triplex PCR for confirmation of fluoroquinolone resistance in *Escherichia coli* uropathogenic isolates(Norbert Lehn Germany ; 1996).

Fluoroquinolones resistance is higher in developing countries than in developed countries. My study shows higher fluoroquinolones resistance rates due to increasing prescriptions without noticing resistant pattern of fluoroquinolones towards urinary *Escherichia coli* as ours is an under developed country(Hafizah Y.chenia ; 2006). Fluoroquinolones resistance is an Independent risk factor for mortality of patients due to delay in the initiation of appropriate antimicrobial therapy for patients with fluoroquinolone resistance infection and now it is no larger significant, after adjusting for inadequate empirical therapy(Ebbing Lautenbach ; etal; 2005).

Fluroquinolones are the drug of choice for urinary tract infections for their high

bacteriological and clinical cure rates as well as low rates of resistance among uropathogens. But due to increased prescriptions more frequently in past few years, increase in fluoroquinolone resistant *Escherichia coli* has occurred which is accounting for significant morbidity and health care cost.

Urine culture and antimicrobial susceptibility testing are essential for patients with urinary tract infections who are having risk factors for resistance occurring such as previous fluoroquinolones usage or inadequate dosage.

Fluoroquinolones antibiotic resistance is becoming a big problem for 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 fluoroquinolones antibiotic prescription policy for urinary tract infections in our country.

Table.1 Antimicrobial susceptibility testing to *Escherichia coli* strains isolated

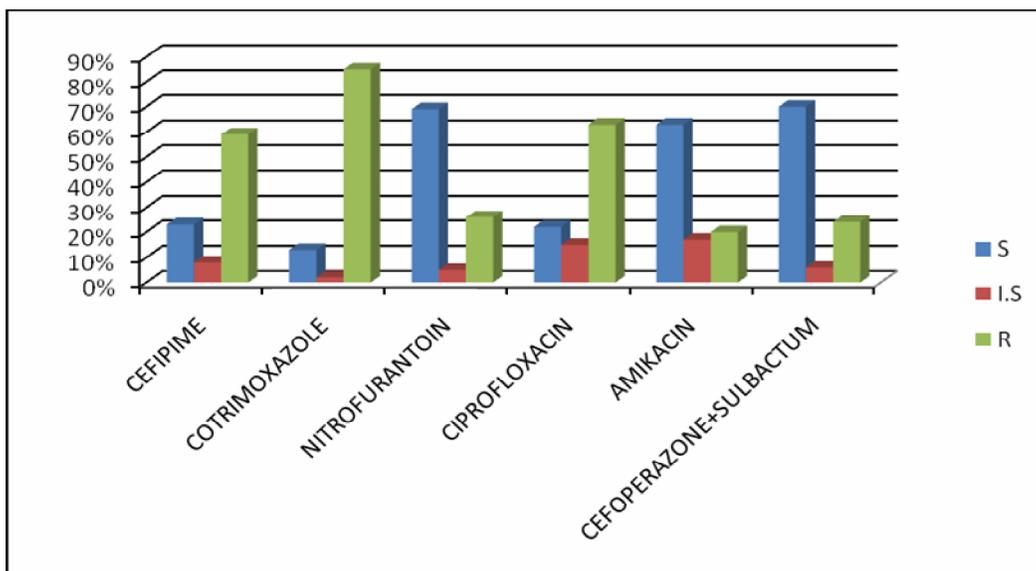
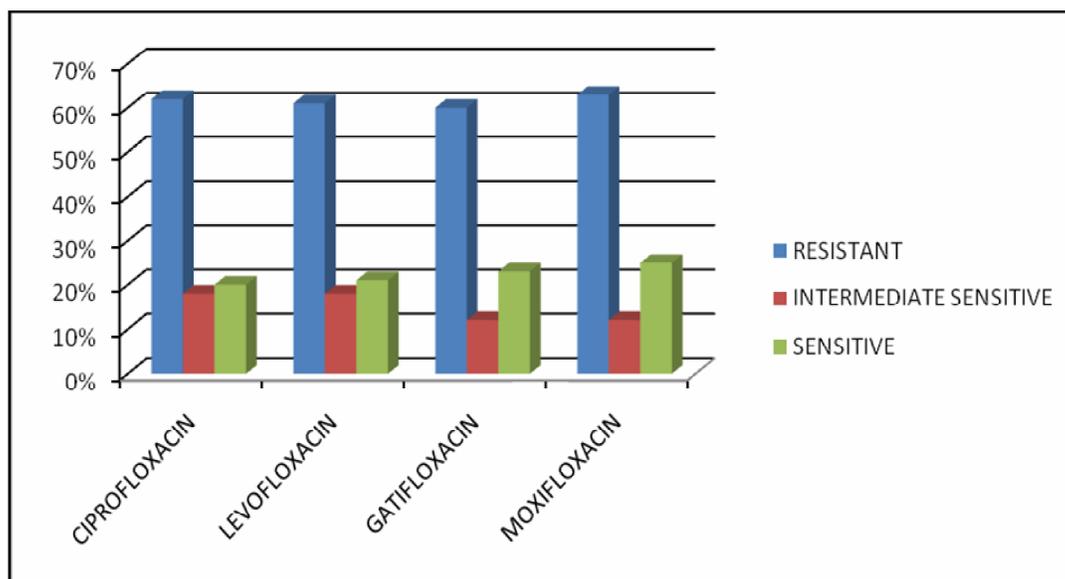


Table.2 Sensitivity of *Escherichia coli* to fluoroquinolones



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