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Research Article

Emerging Ciprofloxacin resistant *Escherichia coli* among urinary tract isolates. Is Ciprofloxacin resistance, a bench marker resistance to other fluoroquinolones?

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Abstract

Introduction: *Escherichia coli (E. coli)* are the common bacteria causing urinary tract infections (UTI's) worldwide. Ciprofloxacillin, a fluoroquinolone, is the most common empirical drug prescribed for urinary tract infections due to *E. coli*. Increased use of ciprofloxacin has resulted in its resistance to *E.coli* and resistance to one generation of fluoroquinolone may also confer resistance to other fluoroquinolones. This study aims to describe the antibiotic susceptibility pattern of E.coli isolates from patients with UTI's. Ciprofloxacin resistant E.coli isolates were analyzed for antibiotic susceptibility to further generation fluoroquinolones.

Material and Methods: A total of 248 urine samples which were positive for E.coli were randomly selected for this study. Antibiotic susceptibility testing of the *E. coli* isolates was done by Kirby – Bauer disc diffusion technique in accordance with the CLSI (Clinical Laboratory Standard Institute). 100 ciprofloxacin resistant E.coli isolates were further studied for their antibiogram to levofloxacin, gatifloxcin and moxifloxacin. The clinical and demographic profile of patients was noted.

Results: High rates of susceptibility was found to cefoperazone + sulbactam (n=175, 70.5%), followed by ciprofloxacin (n=144, 58%), cefipime (n=142, 57.2%), nitrofurantoin (n=142, 57.2%) and amikacin (n=138, 55.6 %). The lowest susceptibility was to trimethoprim-sulphomethoxazole (n=30, 12%). Among these 100 ciprofloxacin resistant isolates there was only a slight improvement in their susceptibilities even to other fluoroquinolones.

Discussion: This study shows emerging ciprofloxacin resistance among urinary tract E.coli isolates. The ciprofloxacin resistant E.coli isolates showed only a slight improvement in their susceptibilities to other fluoroquinolones.

Conclusion: Fluoroquinolones may be prescribed following culture and sensitivity results and not as empirical drugs. This study recommends nitrofurantoin for empirical therapy in UTI. It also suggests that ciprofloxacin resistance could be considered as a bench marker for resistance to other fluoroquinolones.

Keywords: Ciprofloxacin, urinary tract infections, ciprofloxacin resistance

1. Introduction

Urinary tract infections are common infections in community practice. Uncomplicated Urinary tract infections (UTIs) are more common in adult women.[1] Antibiotic resistance among bacteria is a worldwide health problem. *E.coli* cause majority of all urinary tract infections and the emergence of antibiotic resistant *E. coli* presents a challenge for urinary tract

health. *E. coli* accounts for 75% to 90% of urinary tract infections.[2] Ciprofloxacin, a fluoroquinolone, showed excellent activity against *E.coli* and is being used as empirical antibiotic of choice for patients with uncomplicated urinary tract infections. Broad spectrum activity and ease of oral administration led to this antibiotic being overused both in the community and in the hospitals.[3] Development of resistance to fluoroquinolones is related to its amount of use.[4] Various studies in the literature emphasized emerging resistance to fluoroquinolones. Prior fluoroquinolone use, long term hospital stay, older age are independent risk factors for fluoroquinolone resistance. In Netherlands fluoroquinolone resistance increased from 1% in 1989 to 6.1% in 1998 because of increase in number of prescriptions.[5] In Gaza strip, it was 4.1% in 2000, 11.3% in 2002 and 15% in 2004.[6]

In China the increase in ciprofloxacin resistance was from 47% to 59.4% among the isolates during 1998- 2002.[7] In United States the increase in resistance was from 3% to 17.1% in *Escherichia coli* isolates from urine samples of outpatient's between 2000 and 2010.[8] Ciprofloxacin trap the enzymes of DNA during topoisomerization reaction, forming a physical barrier to the movement of replication of RNA polymerase as DNA helicase. This triggers the poorly defined events within the cell that ultimately result in cell death.[9] Fluoroquinolone resistance among *Escherichia coli* is chromosomally mediated.[10,11] The resistance may be due to mutations in gyr A gene that codes for DNA gyrase and par C gene that codes topoisomeraseIV enzymes which alters quinolone enzymatic targets. Other causes of resistance are decrease in bacterial outer membrane permeability for entry of the drug or due to over development of endogenous multi drug resistant efflux mechanisms, wherein the drug is actively pumped out of the bacteria with reduced accumulation in the bacteria[12,13].

Target site mutations have a more significant role in the development of resistance. Molecular studies revealed that a single mutation may rise the MIC of ciprofloxacin to 4 to 16 fold.[14] Low level fluoroquinolone resistance in urinary *Escherichia coli* isolates is associated with single mutation (serine-83 to leucine) in gyrase A position and high level resistance is due to double mutation in gyrase A (serine-83 to leucine and aspartate-87 to Glycine).[15] As mechanism of action of all fluoroquinolones is similar, resistance to one generation may result in resistance to further generation fluoroquinolones. Emergence of fluoroquinolone resistance among *Escherichia coli* isolates, restricts its use as an empirical drug of choice in community acquired UTIs. Its empirical administration with out sensitivity results may cause treatment failures and increased mortality due to urosepsis.

So it is the time to look for other available options to replace fluoroquinolones as an empiric drug of choice in UTI caused by *Escherichia coli* in the community. This study was conducted to assess the current antibiotic resistance pattern of urinary isolates of *Escherichia coli* in patients with uncomplicated urinary tract infections in a teaching hospital in south India. This study also suggests an empirical drug for uncomplicated UTIs. The mechanisms of bacterial resistance to fluoroquinolones fall into two principal categories, alterations in drug target enzymes and alterations that limit permeation of drug to the target, both resulting from chromosomal mutations. As the mechanism of developing resistance among all fluoroquinolones is similar, an attempt was made to establish that ciprofloxacin resistance could constitute a bench marker for resistance to other fluoroquinolones.

2. Material and Methods

2.1 Sample collection and processing

This study was carried out over a period of 6 months between November 2008 and May 2009, at Narayana Medical College and Hospital at Nellore, Andhra Pradesh, India. Appropriate approval was obtained from local ethics committee. The nature of the study was fully explained to all patients and the study was conducted with their informed consent. The urine samples were analyzed in the department of microbiology at Narayana Medical College and Hospital, Nellore.

A total of 248 urine samples which were positive for *Escherichia coli* isolates were randomly selected for this study. The samples were collected from both inpatients and outpatients and included male and female patients between 16 to 65 years of age who attended the hospital. Samples were collected from patients with clinical diagnosis of uncomplicated urinary tract infection and who have not received antibiotics within 3 days of presentation to the hospital. Patients demographics including age, sex and previous antibiotic usage and relevant medical history were collected from information provided to the laboratory and from patients' clinical notes. Exclusion criteria for the study included pregnant and lactating women, patients who had genito-urinary tract disease or abnormalities that may preclude evaluation of therapeutic response or those who had gastrointestinal tract conditions that might affect adequate drug absorption.

Clean voided midstream urine samples were collected. Pyuria was considered if there was ≥ 10 leucocytes/ml of centrifuged urine sample. Only one isolate per patient was processed to avoid strain duplication. Samples were processed on the same day and when there was delay; samples were stored at 2°C- 4°C until being processed. A semi quantitative method

was adopted for primary isolation of organisms using a calibrated loop of 4 mm diameter which delivers 0.01ml of urine.[16] The specimens were inoculated on nutrient agar, blood agar, and MacConkey agar plates and incubated aerobically at 37°C for 24-48 hours. Culture plates with colony counts of $\geq 10^5$ colony forming units (CFU) were considered positive for UTI. Cultures that showed no growth in 24 to 48 hours indicated absence of infection. Initial characterization of E.coli was based on colony morphology, Grams staining and confirmation was done by standard biochemical reactions.[17]

2.2 Antibiotic susceptibility testing:

Antibiogram of 248 urinary isolates of *Escherichia coli* was done by Kirby – Bauer disc diffusion technique on Mueller-Hinton agar media in accordance with the CLSI.[18] Quality controls employed standard strains of *Escherichia coli* ATCC 25922.[17] For this study, we present susceptibility data for cefipime 30µg; trimethoprim-sulphomethoxazole1.25/23.75µg; nitrofurantoin 300µg; amikacin 30µg; cefoperazone plus sulbactum 75/30µg and ciprofloxacin 5µg in the first instance. The *Escherichia coli* which were resistant to ciprofloxacin were further analyzed for their susceptibility to levofloxacin 5µg; gatifloxcin 5µg and moxifloxacin 5µg.

3. Results

A total of 248 urine samples which were positive for *Escherichia coli* isolates were randomly selected for this study and antibiotic susceptibility testing was carried out for cefipime, trimethoprim-sulphomethoxazole, nitrofurantoin, amikacin, cefoperazone plus sulbactam and ciprofloxacin. These *Escherichia coli* isolates showed wide differences in their susceptibility to the tested antimicrobial agents and this is summarized in Table 1. High rates of susceptibility was found to cefoperazone plus sulbactam (n=175, 70.5 %), followed by ciprofloxacin (n=144, 58%), cefipime (n=142, 57.2%), nitrofurantoin (n=142, 57.2%) and amikacin (n=138, 55.6%). The lowest susceptibility was to trimethoprim-sulphomethoxazole (n=30, 12%). Among these 248 *Escherichia coli* isolates, more resistance was observed to trimethoprim – sulphomethoxazole (n=185, 74.59%) followed by ciprofloxacin (n=100, 40%) nitrofurantoin (n=84, 33.8%), amikacin (n=70, 28%), cefoperazone plus sulbactam (n=58, 23%) and lowest resistance was to cefipime(n=20, 8%).

Table1: Cefipime, Trimethoprim/sulfamethoxazole, nitrofurantoin, ciprofloxacin, amikacin and cefoperazone plus sulbactum susceptibilities for 248 urine isolates of *E. coli.*

S. No	Cefepime	Trimethoprim / sulfamethoxazole,	Nitrofurantoin	Ciprofloxacin	Amikacin	Cefoperozone + sulbactum
Sensitive	142	30	142	144	138	175
Intermediate Sensitive	86	33	22	4	40	15
Resistant	20	185	84	100	70	58

Table 2 shows antibiogram of 100 ciprofloxacin resistant *Escherichia coli* isolates to levofloxacin, gatifloxcin and moxifloxacin. Interestingly maximum resistance was observed to gatifloxacin (n=65) followed by moxifloxacin (n=63) and levofloxacin (n=61). Also among these 100 ciprofloxacin resistant isolates there was only a slight improvement in their susceptibilities even to other fluoroquinolones. Among them only 21, 23 and 25 isolates were sensitive to levofloxacin, gatifloxacin and moxifloxacin respectively.

Table 2: Levofloxacin, gatifloxacin and moxifloxacin susceptibilities for 100 isolates of *E. coli* with ciprofloxacin resistance.

S. No	Levofloxacin	Gatifloxacin	Moxifloxacin
Sensitive	21	23	25
Intermediate Sensitive	18	12	12
Resistant	61	65	63

Among the 248 *Escherichia coli* isolates 153 (61%) isolates were from female patients and 95 (38%) were from male patients. Further observations showed that among the 144 ciprofloxacin sensitive isolates 84 (58%) were isolated from female patients and 60 (41%) were from male patients. Also among the 100 ciprofloxacin resistant *Escherichia coli* isolates 67 (67%) were isolated from females and 33 (33%) were from males. In this study the observed fluoroquinolones resistance ratio between females and males was 2.03:1.Of the 144 ciprofloxacin sensitive isolates 94 (65%) were outpatients from the community and 50 (35%) were inpatients from the hospital. However among the 100 ciprofloxacin resistant isolates 52 (52%) were from inpatients and 48 (48%) were from outpatients from the community with urinary tract infections.

Among these 100 ciprofloxacin resistant *Escherichia coli*, 52 were isolated from urology department, 30 were from the department of obstetrics and gynaecology, 16 from general medicine department and only 2 were isolated from endocrinology department. With reference to age, seventy nine percent of ciprofloxacin resistant isolates were observed between 36 to 65 years of age and only 21% of ciprofloxacin resistant isolates were observed between 16 to 35 years of age.

4. Discussion

Antibiotic resistance among bacteria is a worldwide health problem. *Escherichia coli* cause majority of all urinary tract infections and the emergence of antibiotic resistant *E. coli* presents a challenge for urinary tract health. Trimethoprim-sulphomethoxazole, cephalosporins, ciprofloxacin and nitrofurantoin are considered as empiric drugs of choice to treat uncomplicated UTIs. Community acquired uncomplicated UTI is a common indication for prescribing antibiotics empirically and increasing prevalence of UTI caused by antibiotic-resistant bacteria makes empirical treatment of these infections more difficult.[19] Empiric drugs should be prescribed based on local antibiotic susceptibility pattern of the isolates.

Trimethoprim/sulfamethoxazole was the recommended treatment of choice for uncomplicated UTI according to the Infectious Diseases Society of America (IDSA) guidelines 1999. [20,21] The IDSA, also recommended that if resistance to Trimethoprim/sulfamethoxazole is greater than 10 to 20%, it should not be considered for first-line empirical therapy.[21,27] In this study, as the resistance to trimethoprim-sulfamethoxazole was observed in 74.5 % of isolates, it may not be recommended for empirical therapy in uncomplicated UTI's. These results were higher compared to a study from Turkey,[20] where trimethoprim/sulfamethoxazole resistance was observed in 36% of the uncomplicated UTIs and 42% of the complicated UTIs. The results from this study were also higher than in previous American (16.1%) and European (14.1%) studies for uncomplicated UTIs[20,22,23]

In the early 2000s, quinolones surpassed sulfa drugs as the most common class of antimicrobials prescribed by clinicians to treat uncomplicated UTI's.[8,24] An association between the increase in quinolone prescriptions and an increase in bacterial resistance has been reported from several different countries.[3,5,20,23] Resistance rates for ciprofloxacin in uncomplicated

UTIs was reported as 0–14.7% in the ECOSENS Project, 2.5% in the USA and 1.2% in outpatients in Canada.[20,22,23,25] Recently, Alos and co-workers reported 8.5% and 19.5% resistance rates for the uncomplicated and complicated UTI strains, respectively.[26] In this study, resistance to ciprofloxacin was observed in 40% (n=100) of *Escherichia coli* isolates, which is higher than the rates quoted by the studies mentioned above. The reason for this high rate of ciprofloxacin resistance may be due to its frequent use, as it is the empirical drug of choice for uncomplicated urinary tract infections. This study recommends ciprofloxacin in the treatment of UTI's following culture and sensitivity testing but not as an empirical drug. Similar recommendations were also made by other studies.[8,27] Similar high rates of ciprofloxacin resistance were observed in a study from Turkey which showed ciprofloxacin resistance of 17% for the uncomplicated UTI's, and 38% for the complicated UTI's.[20]

Cefoperazone, which acts by inhibiting the bacterial cell wall, is a third generation cephalosporin. In this study, cefoperazone plus sulbactum (a beta lactamase inhibitor) combination has showed resistance in 23% (n=58) of isolates and could be considered as empirical drug in UTI's. However its cost, route of administration, renal toxicity, observed allergy in some individuals, limits its use as an empirical drug in uncomplicated UTIs. Further, because of its antipseudomonal activity and the need to be considered as an option in complicated UTI's and in other Gram negative septicemias it may not be recommended as an empirical drug of choice for uncomplicated UTIs. In this study, resistance to amikacin was observed in 28 % (n=70) isolates. In view of its potential renal toxicity, route of administration and the need to administer in multiple daily doses and the necessity for regular renal monitoring, it may not be considered as an empirical drug in uncomplicated UTIs. In this study only 8% (n=20) of E.coli isolates showed resistance to cefepime, a potent cephalosporin and may be considered as an empirical drug in uncomplicated UTIs. However because of its broad spectrum activity with enhanced activity against both Gram positive and Gram negative organisms, it should be reserved for the treatment of complicated urinary tract infections, intra abdominal infections, urosepsis and in other Gram negative septicemias.[28]

Nitrofurantoin is well absorbed orally and is rapidly excreted in the urine so that drug levels in urine are high with minimal serum levels. Ease of oral administration, minimal side effects, availability in different strengths makes nitrofurantoin to be considered as an empirical drug for UTI's. Due to its combination of multiple sites of attack and multiple mechanisms of action, development of antibacterial resistance to nitrofurantoin is rare and makes it as a better empirical antibiotic of choice in uncomplicated UTIs.[29] In a study from Turkey, resistance rates for the study population

were 4% for nitrofurantoin.[20] In this study, nitrofurantoin resistance was 34% which was higher than previously published data.[22,23,27] However considering its pharmacokinetics, this study recommends nitrofurantoin to be used as empirical drug in uncomplicated UTIs. Randomized clinical trial data from the literature also provide evidence supporting the role of nitrofurantoin as an empirical drug in UTIs.[27]

Table 2 shows antibiogram of 100 ciprofloxacin resistant *Escherichia coli* isolates to levofloxacin, gatifloxcin and moxifloxacin. Interestingly, there was only a slight improvement in susceptibilities to the other fluoroquinolones. Among these 100 ciprofloxacin resistant isolates only 21, 23 and 25 isolates were sensitive to levofloxacin, gatifloxacin and moxifloxacin respectively. These results may support the suggestion that resistance to ciprofloxacin could be considered as a bench mark for resistance to other fluoroquinolones. Similar such results were also observed in the literature with nalidixicacid.[30]

In this study, among the 248 *Escherichia coli* isolates 153 (61%) isolates were from female patients and 95 (38%) were from male patients The observed fluoroquinolones resistance ratio between females and males is 2.03:1. Uncomplicated urinary tract infections are common in adult women[1] and frequent use of ciprofloxacin for recurrent UTIs contributes to the increase in ciprofloxacin resistance among women. In the Duke study 1.8% ciprofloxacin resistance among women with no prior UTI, rose to 11.8% in a recurrent UTI. Similar such study in Taiwan found that prior exposure to ciprofloxacin raises the risk for resistance by a factor of 13.[21,31] With reference to age, in the present study, 79% of ciprofloxacin resistant *Escherichia coli* isolates were observed between 36 to 65 years of age and only 21% of ciprofloxacin resistant isolates were observed between 16 to 35 years of age. This may be due to decreased immune function and more frequent use of fluoroquinolones with increase in age.[32] Among the 100 ciprofloxacin resistant *E. coli* isolates 52 were isolated from urology department, 30 were from obstetric and gynecology department, 16 were medicine department and 2 were isolated from endocrinology department. This may be because of increased exposure to infections in the departments involving surgical procedures and medical interventions in certain departments.

Overall antibiotic susceptibility testing in this study demonstrates increased resistance to many commonly used antimicrobial agents and illustrates the need for continuous evaluation of the common antibiotics used in the treatment of urinary tract infections.

5. Conclusion

There is increase in ciprofloxacin resistance among urinary isolates of *Escherichia coli*. Fluoroquinolones should only be prescribed following culture and sensitivity testing but not for empirical therapy. Our study recommends nitrofurantoin, for empirical therapy in uncomplicated UTIs. Further this study also suggests that ciprofloxacin resistance may constitute a bench marker for resistance to further generation fluoroquinolones in the routine laboratories. Last but not the least, we need to be aware that patient needs and clinical correlation will play vital role in the empirical therapy of individual patient with urinary tract infections.

References

1. Mohsin R, Siddiqui KM. Recurrent urinary tract infections in females. *J Pak Med Assoc*. 2010; 60(1): 55-9.
2. Gupta K. Addressing antibiotic resistance. Dis Mon. 2003;49(2):99–110.
3. Norrby SR, Lietman PS. Safety and tolerability of fluoroquinolones, *Drugs*. 1993; 45 Suppl 3:59-64.
4. Zervos MJ, Hershberger E, Nicolau DP, Ritchie DJ, Blackner LK, Coyle EA, *et al*. Relationship between fluoroquinolone use and changes in susceptibility to fluoroquinolones of selected pathogens in 10 United States teaching hospitals, 1991–2000. *Clin Infect Dis*. 2003;37(12):1643-8.
5. Goettsch W, van Pelt W, Nagelkerke N, Hendrix MG, Buiting AG, Petit PL, *et al.* Increasing resistance to fluoroquinolones in *Escherichia coli* from urinary tract infections in The Netherlands. *J Antimicrob Chemother.* 2000;46(2):223-8.
6. Zakaria El Astal. Increasing ciprofloxacin resistance among prevalent urinary tract bacterial isolates in Gaza strip. *J Biomed Biotechnol.* 2005; 2005(3): 238–241.
7. Hooper D.C. Emerging mechanisms of fluoroquinolone resistance. *Emerg Infect Dis* 2001; 7:337-41.
8. Guillermo V. Sanchez, Ronald N. Master, James A. Karlowsky, and Jose M. Bordon. In Vitro Antimicrobial Resistance of Urinary *Escherichia coli* Isolates among U.S. Outpatients from 2000 to 2010. *Antimicrobial Agents and Chemotherapy*. 2012; 56(4): 2181–2183.

9. David C Hooper. Emerging mechanism of fluoroquinolone resistance. *Drug resistance-original article*.1999; 2: 38 – 55.
10. Patricia KL, Karl soon A, Hughes D. Mutation rate and evaluation of fluoroquinolone resistance in *Escherichia coli* on the aspect of gyrase and multiple antibiotic resistance genes. *J Antimicrobial Agents Chemother.* 2003; 47: 3222 –32.
11. Friedman T, Drlica K. Mutations in DNA gyrase 'A' gene of *Escherichia coli* that expand the quinolone résistance – determine region. *J Antimicrob Agents Chemotherapy*. 2001; 45: 2378 –80.
12. Heisig P. Genetic evidence for a role of *Par*C mutations in development of high-level fluoroquinolone resistance in *Escherichia coli*. *Antimicrob Agents Chemother* 1996;40:879-85.
13. Hooper D.C. Emerging mechanisms of fluoroquinolone resistance. *Emerg Infect Dis* 2001;7:337-41.
14. Shao HF, Wang WP, Zhang XW, Li ZD Distribution and resistance trends of pathogens from urinary tract infections and impact on management. *Zhonghua Nan Ke Xue*. 2003 Dec;9(9):690-2, 696.
15. Mc Donald L Clifford, Feing-jui-chen. Emergence of reduced susceptibility and resistance to fluoroquinolone in *Escherichia coli* in Taiwan and contributions of distinct Selective pressure. *J Antimicrob Agents Chemother.* 2001; 45(11):3084-91.
16. Ozlem KA Hande A. Risk factors for ciprofloxacin resistance among *Escherichia coli* strains isolated from community acquired urinary tract infections in turkey. *J Antimicrobial Chemother.* 2005; 56(5):914-918.
17. Mackie McCartney. Practical medical microbiology. 1st Edition reprint: p135-136.
18. CLSI M100-S20 - Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement, M100S20 Wayne, PA:Clinical and Laboratory Standards Institute; 2010:40-51 and 135.
19. Steinke DT, Seaton RA, Philips G et al. Prior trimethoprim use and trimethoprim-resistant urinary tract infection: a nested case–control study with multivariate analysis for other risk factors. *J Antimicrob Chemother* 2001; 47: 781–7.
20. Arsian H, Azap OK, Ergonul O, Timurkaynak F. Risk factors for ciprofloxacin resistance among *Escherichia coli* strains isolated from community-acquired urinary tract infections in Turkey. *J Antimicrob Chemother.* 2005; 56(5):914-8.
21. Warren JW, Abrutyn E, Hebel JR, Johnson JR, Schaeffer AJ, Stamm WE. Guidelines for the treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. *Clin Infect Dis* 1999; 29(4):745-58.
22. Karlowsky JA, Kelly LJ, Thornsberry C, Jones ME, Sahm DF.Trends in antimicrobial resistance among urinary tract infection isolates of *Escherichia coli* from female outpatients in the United States. *Antimicrob Agents Chemother.* 2002 Aug;46(8):2540-5.
23. Kahlmeter G. An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO.SENS Project. *J Antimicrob Chemother* 2003; 51: 69-76.
24. Kallen AJ, Welch HG, Sirovich BE. 2006. Current antibiotic therapy for isolated urinary tract infections in women. *Arch. Intern. Med*. 166: 635-639.
25. Zhanel GG, Karlowsky JA, Harding GK, Carrie A, Mazzulli T, Low DE, et al. A Canadian national surveillance study of urinary tract isolates from outpatients: comparison of the activities of trimethoprim- sulfamethoxazole, ampicillin, mecillinam, nitrofurantoin and ciprofloxacin. *Antimicrob Agents Chemother.* 2000;44(4):1089-92.
26. Alos JI, Serrano MG, Gómez-Garcés JL, Perianes J. Antibiotic resistance of *Escherichia coli* from community-acquired urinary tract infections in relation to demographic and clinical data. *Clin Microbiol Infect*. 2005 Mar;11(3):199-203.
27. Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG,et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. *Clin Infect Dis*. 2011 1;52(5):e103-20.
28. Sukhbir K. Shahid. Cefepime and its Role in Pediatric infections. Recent Patents on Anti-Infective *Drug Discovery 2008;* 3 (2): 145-148.
29. McOsker CC, Fitzpatrick PM. Nitrofurantoin:mechanism of action and implications for resistance development in common uropathogens. *J Antimicrob Chemother.* 1994; 33 Suppl A:23-30.
30. Carmen Antonia Sanches ItoI, IV; Ana Cristina GalesII; Maria Cristina B. TognimII; Patrícia MuneratoIII; Libera Maria Dalla Costa I V. Quinolone-resistant *Escherichia coli*. *Braz J Infect Dis* 2008; 12 (1) Salvador
31. Linda Fugate, Antibiotic Resistant *E. coli* Urinary Tract Infection, HER Writer November 9, 2009
32. Lauren becnel Boyd, Robber atnam L, Graven L, Richard J, David Steffen. Increased Fluoroquinolones resistance with time in *Escherichia coli* from 17,000 patients at a large country hospital as a function of culture site, age, sex and location. *J BMC Infect Diseases*. 2008; 8: 71.