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Short Communication

Antimicrobial Susceptibility Testing for *Escherichia coli* Strains to Fluoroquinolones, in Urinary Tract Infections

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Abstract

Background: Urinary Tract Infections (UTIs) are one of the most common infectious diseases diagnosed all over the world. Meanwhile most episode of UTIs are caused by *Escherichia coli* (up to 85%) and frequently fluoroquinolones are preferred as initial agents for empiric therapy of UTIs. Widespread use of fluoroquinolones has resulted in an increasing incidence of resistance these agents all over the world. The aim of this study was to assess, susceptibility of *Escherichia coli* strains from UTI patients against common fluoroquinolones. **Methods:** Antimicrobial susceptibility testing was determined by disk agar diffusion (DAD) and Minimal Inhibitory Concentration methods as described by the National Committee for Clinical Laboratory Standards (NCCLS). **Results:** One hundred sixty four clinical isolates of *E. coli* were collected by urine cultures from patients with UTI. The extent of resistant to nalidixic acid, ofloxacin, norfloxacin and ciprofloxacin, by disk diffusion method was 49.3%, 44.5%, 41.4% and 40.2%, respectively. Resistance to ciprofloxacin by MIC method was 4.9%. **Conclusion:** This study represents high level resistant of *E. coli* isolates from UTI patients. It is because of inappropriate and incorrect administration of antimicrobial agents in blind cases. This problem remarks significance of performing antimicrobial susceptibility testing before empiric antibiotic therapy. To overcome this problem use of unnecessary antibiotics therapy should be limited.

Keywords: *Urinary tract infection, UTI,* E.coli*, Fluoroquinolones, Resistance, Iran*

Introduction

Urinary tract infections (UTIs) represent one of the most common diseases encountered in medical practice today. It is estimated that about 20-30% of adult women experience UTI at least once during their life (1). However, its impact and frequency vary in different populations. UTIs occur at a rate of 2-3 per 100 hospital admissions and constitute 35-40% of all hospital-acquired infections (2). Most episodes of UTIs are caused usually by *Escherichia coli* (75% to 90%) (3). Often fluoroquinolones are preferred as initial agents for empiric therapy of UTIs. Ciprofloxacin is the most frequently prescribed fluoro-quinolones for UTIs because it has shown excellent activity against pathogens commonly encountered in complicated UTIs (4). Resistance to fluoroquinolones has increased markedly since their introduction for UTI treatment. Many studies worldwide reported a noticeable increase in ciprofloxacin and other fluoroquinolones resistance (5). Consequently, the extensive uses of antimicrobial agents have invariably resulted in the development of antibiotic resistance, which, in recent years, has become a major problem worldwide (6).

Materials and Methods

One hundred sixty four clinical isolates of *E. coli* were collected by urine cultures from patients with UTI in Sina, Shariati and Children Medical Center in Tehran, Capital of Iran, from May to September 2005. The colony count of these cultures was $\geq 10^5$ bacteria/ml and ≥ 10

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WBC/hpf. They were identified by routine laboratory methods.

Antimicrobial susceptibility testing was determined by disk agar diffusion method (DAD) as described earlier (7). For susceptibility data collection 30µg of nalidixic acid, 5µg of ofloxacin, 10µg of norfloxacin and 5µg ciprofloxacin per disk were used. Minimal Inhibitory concentrations were performed and results were compared by broth micro dilution method for ciprofloxacin as described by earlier (7). The minimal inhibitory concentrations (MICs) were defined as the lowest antimicrobial concentration which was able to inhibit bacterial growth with final inoculum's of 10^6 CFU. All plates were incubated aerobically at 37 ºC overnight.

The MICs were determined by 2-fold serial dilution in cation-adjusted Muller Hinton broth. Antibiotic concentrations in micro plates were 0.25 to 128µg/ml. *Escherichia coli* ATCC 25922 was used as standard strain according to NCCLS protocols (7). Fluoroquinolone discs and ciprofloxacin powder were obtained from pharmaceutical incorporations (MAST. Inc, UK). Cation-adjusted Muller Hinton broth was supplied by Merch Inc, Germany.

Results

Forty seven percent of *E. coli* isolates were collected from Shariati Hospital, 25% from Sina Hospital and 28 % from Children Medical Center of Tehran. Of all 164 samples 35.3% were from males. The extent of resistant to nalidixic acid, ofloxacin, norfloxacin and ciprofloxacin, by disk diffusion method were 49.3%, 44.5%, 41.4% and 40.2 %, respectively. Approximately similar trends were observed for sensitivity pattern of norfloxacin and ofloxacin. Resistance to ciprofloxacin by MIC method, 4.9% had reduced susceptibility and 53% remained susceptible.

Discussion

The fluoroquinolones are a highly active class of agents which are effective in treating different infections. The use of antibiotics in the treatment and prevention of infectious diseases is clearly a necessity, however much care must be given to the choice of antimicrobial agents to be used. (8, 9) Unfortunately urinary tract isolates are becoming increasingly resistant to commonly used fluoroquinolones.

Moreover, in patients with suspected UTI, antibiotic treatment is usually started empirically, before urine culture results are available. To ensure appropriate treatment, knowledge of the organisms that cause UTI and their antibiotic susceptibility is mandatory (10, 11).

In the present study, 164 isolates of *E.coli* were analyzed in term of antibiotic susceptibility. According to the demographic data, females are affected more often than males. The significant differences in UTI rates between females and males are thought to be due to anatomical differences between the sexes. The quinolones are themselves mutagenic. One suggestion is that they induce mutations through the generation. To address this idea, quinolone mutagenicity has been examined by the Ames test (12).

In this study, 40.2% of *E.coli* isolates from urine samples were resistant to ciprofloxacin and 49.3% to nalidixic acid. Moreover this study shows generalized decrease in bacterial susceptibility of *E. coli* to common fluoroquinolones. It could be concluded empirically that use of fluoroquinolones in blind cases reduces the susceptibility of *Escherichia coli.* The persistent decrease in sensitivity of *E. coli* to fluoroquinolones is worrying, because these antibiotics have proven very effective for the treatment of UTI in both, hospitalized and outpatients (13).

Comparison of our results with findings from previous studies in different countries showed higher resistance. For example in Turkey, the incidence of resistance of *E. coli* from urinary cultures to quinolones increased from 6.4% in 2003 to 21% in 2000 (14).

Similar findings have been reported in other countries. For example in the year 2003 in Kuwait the extent of resistance was 17.8% (5) and in Gaza stripe in the year of 2005 it was 17.5%

(4). In this investigation, 42.1% of *E. coli* isolates from UTIs in patients were resistant to ciprofloxacin and 4.9% of them had reduced susceptibility. Therefore, the finding of such large resistance population of *E. coli* (41.2% resistant and 4.9% with reduced susceptibility) isolates from Tehran suggests that the rate of resistance may rapidly increase further.

Notably, comparison among different studies concerning resistance of uropathogenes to different antimicrobial agents should take in to account the different periods in which such studies were carried out as well as various socioeconomical, socioepidemiological and clinical parameter of target population. Moreover the comparison must consider the limitation of resistance to antimicrobial which can vary from country to another.

In conclusion, this high level-resistance to fluoroquinolones in patients with UTI should be important to a possible cause of the increase widespread use of these agents in medical centers. There are many reasons for this alarming phenomenon, including inappropriate prescribing of antibiotics and poor infection control strategies (4, 5, 14). To overcome this problem the use of unnecessary antibiotics therapy should be limited.

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