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**ORIGINAL ARTICLE**

# Prevalence of Ciprofloxacin Resistance among Gram-Negative Bacilli Isolated From Urinary Tract Infection Specimens at a Specialist Hospital in Riyadh, Saudi Arabia

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**Abstract**

**Background:** Susceptibility to fluoroquinolones is very important information before prescribing to the urinary tract infection (UTI) patients. **Objective:** The purpose of the present study was to see the sensitivity pattern of the Gram-Negative bacilli isolated from urinary tract infection to various antibiotics as well as to know the prevalence rate of Ciprofloxacin resistance. **Methodology:** This cross sectional study was conducted in a specialized hospital in Riyadh, Saudi Arabia from January'2006 to June'2006 for over a period of six months. The Gram-negative bacilli (GNB) were isolated from clinical specimens of UTIs and antibiotic susceptibility testing was performed. Isolates with resistance or with a decreased susceptibility to Ciprofloxacin (20 mm) were than screened for their minimum inhibitory concentration (MIC) by using the E-test. **Results:** Out of 510 GNB, 97(19%) isolates were resistant to Ciprofloxacin. The MIC of these isolates ranged from 4 to 32µg/ml. Most of the Ciprofloxacin resistant isolates were from urinary tract infections (UTI) of hospital patients both (indoor & outdoor). The Ciprofloxacin resistance was also closely associated with multi-drug resistance, thus limiting the treatment options. **Conclusion:** The considerably high MIC values for Ciprofloxacin in this study reflected the extent of the treatment problems for these resistant isolates.

**Keywords:** Gram-negative bacilli, MIC, fluoroquinolone, ciprofloxacin

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## Introduction

There were major therapeutic advance of fluoroquinolone antimicrobials in 1980, because they have 100 fold greater activities than their parent compound called Nalidixic acid (Manno et al.,

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2005). Unlike Nalidixic acid, which is used only for urinary infections and occasionally for shigellosis, the fluoroquinolones have a broad range of therapeutic indications as well as are given as prophylaxis (Rahman 2011). In veterinary medicine fluoroquinolones are used as treatment and metaphylaxis; however, it is not used as growth promoters (Bauernfeind and Petermuller 1983). Early researchers thought that fluoroquinolones resistance was unlikely to evolve, largely because resistant *E. coli* mutants are exceptionally difficult to select in vitro and because plasmid-mediated quinolone resistance remained unknown even after 30 years of Nalidixic acid usage (Martinez-Martinez et al., 1998). Nevertheless multi-national quinolone resistance emerges in *Staphylococci* and *Pseudomonads*, which are inherently less susceptible than *E. coli* (Smith 1996).

More recently, fluoroquinolone resistance has emerged in *E. coli* and other Enterobactericeae which was due to multiple mutations that diminish the affinity of its topisomerase II and IV targets in varying ways; therefore, reduce permeability and up regulate efflux (Ericsson and Sherris 1971). Plsmid-mediated quinolone resistance has been reported (Ahmad 2012); however, it is exceptional. Ciprofloxacin is an antibiotic which is used in UTIs & active against gram-negative bacteria, which belongs to the fluoroquinolone class (Drlica and Zhao 1997). Bacterial resistance is a growing therapeutic problem, both in the community and in the hospitals, involving all the antibiotics, which include fluoroquinolones (Astal 2005). A decreased susceptibility to fluoroquinolones arises mainly due to a single-step mutations in the gyr A & the parC genes, which encode the fluoroquinolone targets, the topisomerase enzymes (Everett et al., 1996). Some mobile elements which were responsible for the horizontal transfer of the quinolone resistance genes were described (Livermore 2009). This study was undertaken to evaluate the susceptibility to GNB to various antibiotics and to know the prevalence rate of ciprofloxacin resistance at a tertiary care hospital in Saudi Arabia.

## Methodology

This cross sectional study was conducted at a tertiary care hospital in Riyadh in Saudi Arabia from January'2006 to June'2006 over a period of six months. All the suspected cases of UTI patients were taken as study population. Mid-stream and morning urine samples were collected by urinary catheters, supra-pubic puncture and were sent to the bacteriology laboratory. Specimens were processed using different media like Sheep Blood Agar, MacConkey's Agar and Cystine Lactose Electrolyte Deficient (CLED) Agar. All isolates were identified by using standard biochemical kits, API 20E (Analytical Profile Index System, La Balme Les Grottes, France), & the fully automated analyzers such as PHOENIX, MICROSCAN & VITEK II were also used for the identification & sensitivity pattern of the pathogens. Antibiotic sensitivity testing was performed mainly by using the fully automated analyzers (Phoenix, Microscan & Vitek II) & also sometimes by using the disc diffusion method on 85 mm Mueller-Hinton Agar (Oxoid) plates with agar depth of 4 mm. The bacterial suspension that was prepared for antibiotic sensitivity testing on Mueller-Hinton Agar or for the fully automated analyzers (Phoenix, Microscan & Vitek II) was adjusted to the recommended turbidities for all species. The antibiotics tested on each disc were Ampicillin (25mg), Amoxicillin-Clavulanic Acid (20-10 mg), Trimethoprim-Sulphomethoxazole (1.25-23.75 mg), Cephalothin (30 mg), Cefuroxime (30 mg), Cefotaxime (30 mg), Cefepime (30 mg), Ciprofloxacin (5 mg), Norfloxacin (30 mg), Nalidixic Acid (30 mg), Gentamicin (10 mg), Amikacin (30 mg), Tazocin

(Piperacillin+Tazobactam), Imipenem (30 mg). The Clinical Laboratory Standards Institute (CLSI) break points were used for interpretation of susceptibility patterns as sensitive & resistant. Isolates with resistance or decreased susceptibility to Ciprofloxacin (20mm) were subjected to further study. The study design & protocol was approved by "Research & Ethics Committee" of the institute. The resistance to Ciprofloxacin was confirmed by break point minimum inhibitory concentration (MIC in µg/ml) by using E-test strips and also by the fully automated analyzers (Phoenix, Microscan & Vitek II). The isolates with MIC value 4 µg/ml were defined as resistant isolates, as outlined by CLSI guidelines.

**Result**

A total of 510 gram-negative bacilli were isolated from clinical specimens of UTIs. Esch. coli (30.5%) was the most predominant isolate which was found among the GNB, followed by Klebsiella pneumoniae (24.5%), Proteus species (16.4%),Pseudomonas aeruginosa (9.2%), Acinetobacter species (7.8%), Enterobacter species (6.2%), Citrobacter species (3.2%), Morganella morganii (1.2%), & Serrtia marcescens (1.0%) (Table 1).

**Table 1: Total number of Gram-Negative Bacilli isolated from clinical specimens of UTIs (n=510)**

| Organism | Frequency | Percentage |
|---|---|---|
| Escherichia coli | 155 | 30.5 |
| Klebsiella pneumoniae | 125 | 24.5 |
| Proteus species | 84 | 16.4 |
| Pseudomonas aeruginosa | 47 | 9.2 |
| Acinetobacter species | 40 | 7.8 |
| Enterobacter species | 32 | 6.2 |
| Citerobacter species | 16 | 3.2 |
| Morganella morganii | 06 | 1.2 |
| Serratia marcescens | 05 | 1.0 |
| **Total** | **510** | **100.0** |

Out of 510 gram-negative bacilli, (97=19%) isolates were resistant to Ciprofloxacin. High rates of resistance were observed for Ampicillin & Cephalothin followed by Amoxicillin-Clavulanic Acid, Trimethoprim-Sulphamethoxazole, while low levels of resistance were observed for Imipenem, Tazocin, Amikacin, Nitrofurantoin,Nalidixic Acid, Gentamicin& Norfloxacin (Table 2). The lowest level of resistance was observed for Tazocin (06%) followed by Imipenem (05%). The resistance rate of Ciprofloxacin was 19%. The MIC of Ciprofloxacin for these isolates ranged from 4 to ≥32µg/ml (Table 3). The isolated bacteria showed wide differences in their susceptibility to Ciprofloxacin. A high rate of resistance to Ciprofloxacin was observed among *P. aeruginosa, Acinetobacter* spp., *K. pneumoniae* AND Proteus spp. followed by E. coli.

**Discussion**

The rapidly rising rates of fluoroquinolone-resistant *E. coli* in many parts of the world have been found due to the reduced susceptibility to the quinolones (Nema et al., 1997). The Surveillance

Network database shows resistance trends in blood-stream isolates from 250 U.S. hospitals which was *E. coli*, 1.8% in 1996 and 4.3% in 1999; *Klebsiella* spp., 7.1% in 1996 and 6.7% in 1999; *Enterobacter* spp., 6.6% in 1996 and 6.5% in 1999; and *Proteus mirabilis*, 5.7% in 1996 and 12.7% in 1999 (Nordmann and Poirel 2005). High rates in *E. coli* may reflect contamination via the food chain; the Spanish study found quinolone-resistant *E. coli* and 90% of chicken feces and noted similar fecal carriage rates of resistant *E. coli* in children and adults (Garau et al., 1999).

**Table 2: Antibiotic susceptibility pattern of isolates to various antibiotics (n=510)**

| Antibiotics | Sensitive isolates | Resistant isolates |
|---|---|---|
| Ampicillin | 82 (16%) | 428 (84%) |
| Cephalothin | 127 (25%) | 383 (75%) |
| Amoxicillin-Clavulanic Acid | 153 (30%) | 357 (70%) |
| Trimethoprim-Sulphamethoxazole | 194 (38%) | 316 (62%) |
| Cefuroxime | 316 (62%) | 194 (38%) |
| Cefotaxime | 331 (65%) | 179 (35%) |
| Cefepime | 382 (75%) | 128 (25%) |
| Norfloxacin | 403 (79%) | 107 (21%) |
| Nitrofurantoin | 408 (80%) | 102 (20%) |
| Nalidixic Acid | 357 (70%) | 153 (30%) |
| Ciprofloxacin | 413(81%) | 97 (19%) |
| Gentamicin | 393(77%) | 117 (23%) |
| Amikacin | 413(81%) | 97 (19%) |
| Tazocin (Piperacillin+Tazobactam) | 479 (94%) | 31 (06%) |
| Imipenem | 484 (95%) | 26 (05%) |

There is a small set of drugs commonly used to treat *P. aeruginosa* infection, including ciprofloxacin, tobramycin, amikacin, gentamicin, ceftazidime, piperacillin, tazocin and imipenem (Hooper 2001). While *P. aeruginosa* has developed various levels of resistance to each of these, its response to Ciprofloxacin is of particular interest because the drug is initially very effective; however, *P. aeruginosa* rapidly acquires high level resistance rendering the drug important (Schaeffer 2002).

The resistance rates for ciprofloxacin were 19% in this present study. Most of the resistant isolates were obtained from UTI samples. This may be because of fluoroquinolones are preferred as the initial agents for empiric therapy in UTI, because of their excellent activity against the pathogens which are commonly encountered in UTI (Schaeffer 2002). This emphasizes the importance of the re-assessment of the antibiotics which are used in the empiric treatment of UTIs. Most of the isolates from UTIs were susceptible to nitrofurantoin, nalidixic acid, amikacin, imipenem. This was in agreement with the finding of a study reported by Astal (2005).

These data suggests that nitrofurantoin can still be successfully used in the treatment of UTI. The ciprofloxacin resistance was also closely associated with multi-drug resistance (Nordmann and Poirel 2005). Hence, it severely limits the already restricted treatment options. The finding was in accordance with the finding of a study which was conducted by Livermore (2009). The high

resistance pattern which was seen in this study was probably due to the inappropriate prescribing of antibiotics sometimes without doing culture and sensitivity tests, lack of antibiotic policy and the poor infection control strategies (Manno et al., 2005). However, the antibiotic history could not be properly elicited from the patients in this study.

**Table 3: MIC values of the resistant Gram-Negative Bacilli to Ciprofloxacin (n=97)**

| Ciprofloxacin MIC values | 4 µg/ml | 8 µg/ml | 16 µg/ml | 24 µg/ml | 32µg/ml |
|---|---|---|---|---|---|
| Total no. of isolates | 19 (20%) | 12 (12%) | 14 (14%) | 15 (15%) | 38(39%) |

Ciprofloxacin remains a potent antibiotic; however, the slow accumulation of resistant *Enterobacteriaceae* is disturbing, not least because resistance is a class effect, affecting all fluoroquinolones (Astal 2005). Ultimately, this resistance may be partly overcome by the efflux pumps that contribute to the resistance but this strategy is still several years from fruition. In the interim, the best approach lies in the prudent use of fluoroquinolones in humans and animals, coupled with an emphasis on preventing patient-to-patient spread of resistant strains (Schaeffer 2002).

The antibiotic which showed maximum activity against most of the isolates was imipenem and tazocin (Manno et al., 2005). Though carbapenems remain the final options for treating these infections, there is a possibility that the increasing use of carbapenems may lead to a rapid emergence of carbapenems resistance. Ciprofloxacin resistance can be used as a general surrogate marker of multi-drug resistance, thus limiting the already restricted treatment options and a need for the continuous evaluation of the commonly used antibiotics.

## Conclusion

The considerably MIC values for Ciprofloxacin, in this study, reflect the scope of limited treatment options which are available for these resistant isolates and a need for the continuous evaluation of the commonly used antibiotics. Repeated surveillance, the formulation of an antibiotic policy, the prudent prescriptions of antibiotics and the recycling of antibiotics are the possible routes which can be used to curb the rapid emergence and the spread of these resistant isolates.

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