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: *Different classes of antimicrobials including fluoroquinolones have shown resistance in a multitude of bacterial species in the hospitals & in the community. Decreased susceptibility to fluoroquinolones arises mainly by single step mutations in the gyrA & parC genes, which encode the fluoroquinolones targets, the topisomerase enzymes conferring cross resistance to all fluoroquinolones. Accumulation to multiple mutations in several genes confers increasing level of resistance associated with clinical failure. However, even low level resistance can generate therapeutic failure. Some mobile elements with a potential for the horizontal transfer of the quinolone resistance genes were described in 1998. The loci which are responsible for this plasmid-mediated quinolone resistance, which have been designated as qnr A, qnr B & qnr S, have been identified in the Enterobacteriaceae species.* ***Aim:*** *To evaluate the susceptibility pattern of the isolates to various antibiotics & to know the prevalence rate of Ciprofloxacin resistance in our hospital.* ***Materials & Methods:*** *A total of 510 gram-negative bacilli (GNB) were isolated from clinical specimens of UTIs over a period of six months (from January'2006 to June'2006) were subjected to antibiotic susceptibility testing. 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 32 µg/ml.* Conclusion: *The resistance rate of Ciprofloxacin was 19% in our study. 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. Ciprofloxacin resistance can be used as a general surrogate marker of multi-drug resistance, thus limiting the already restricted treatment options. The considerably high MIC values for Ciprofloxacin in this study reflected the extent of the treatment problems for these resistant isolates & a need for the continuous evaluation of the commonly used antibiotics.*

Key Words: *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, Nalidixic acid. Unlike Nalidixic acid, which is used only for urinary infections & occasionally for shigellosis, the fluoroquinolones have a broad range of therapeutic indications & are given as prophylaxis, e.g., in veterinary medicine fluoroquinolones are used as treatment. Early researchers thought that fluoroquinolones resistance was unlikely to evolve, largely because resistant Esch.coli mutants are exceptionally difficult to select in vitro & because plasmid-mediated quinolone resistance remained unknown even after 30 years of Nalidixic acid usage. Nevertheless multi-national quinolone resistance emerge in Staphylococci & Pseudomonads, which are inherently less susceptible than Esch.coli. More recently, fluoroquinolone resistance has emerged in Esch.coli & other Enterobactericeae, contingent on multiple mutations that diminish the affinity of its topisomerase II & IV targets in varying ways reduce permeability & up regulate efflux. Plsmid-mediated quinolone resistance has been reported but it is exceptional.

Ciprofloxacin is an antibiotic which is used in UTIs & active against gram-negative bacteria, which belongs to the fluoroquinolone class. Bacterial resistance is a growing therapeutic problem, both in the community & in the hospitals, involving all the antibiotics, which include fluoroquinolones. A decreased susceptibility to fluoroquinolones arises mainly due to a single-step mutations in the gyr A & the parC genes, which encode the

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fluoroquinolone targets, the topisomerase enzymes. In 1998, some mobile elements which were responsible for the horizontal transfer of the quinolone resistance genes were described. This study was undertaken to evaluate the susceptibility to GNB to various antibiotics & to know the prevalence rate of Ciprofloxacin resistance in our hospital.

Materials & Methods:

A total of 510 gram-negative bacilli were isolated from clinical specimens of UTIs i.e., urine samples (mid-stream & morning sample), from urinary catheters, supra-pubic puncture & was received in the Bacteriology Laboratory over a period of six months (January'2006 to June'2006) were subjected to the study. Specimens were processed using different media like Sheep Blood Agar, MacConkey's Agar& 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 (20 mm) were considered as resistant & 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 & also by the fully automated analyzers (Phoenix, Microscan & Vitek II). The isolates with MIC value >32 mg/ml were defined as resistant isolates, as outlined by CLSI guidelines.

Results:

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%) as shown in Table-I.

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-III)

The isolated bacteria showed wide differences in their susceptibility to Ciprofloxacin. A high rate of resistance to Ciprofloxacin was observed among Ps.aeruginosa, Acinetobacter spp., K.pneumoniae & Proteus spp. followed by Esch.coli.

Table-I

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

SL No.	Antibiotics	Total no of Sensitive isolates %	Total no of Resistant isolates %
1.	Ampicillin	82 (16%)	428 (84%)
2.	Cephalothin	127 (25%)	383 (75%)
3.	Amoxicillin-Clavulanic Acid	153 (30%)	357 (70%)
4.	Trimethoprim-Sulphamethoxazole	194 (38%)	316 (62%)
5.	Cefuroxime	316 (62%)	194 (38%)
6.	Cefotaxime	331 (65%)	179 (35%)
7.	Cefepime	382 (75%)	128 (25%)
8.	Norfloxacin	403 (79%)	107 (21%)
9.	Nitrofurantoin	408 (80%)	102 (20%)
10.	Nalidixic Acid	357 (70%)	153 (30%)
11.	Ciprofloxacin	413(81%)	97 (19%)
12.	Gentamicin	393(77%)	117 (23%)
13.	Amikacin	413(81%)	97 (19%)
14.	Tazocin (Piperacillin+Tazobactam)	479 (94%)	31 (06%)
15.	Imipenem	484 (95%)	26 (05%)

Table-II

Antibiotic susceptibility pattern of isolates to various antibiotics (n=510)

SL No.	Organism	Total no of isolates	Percentage (%)
1.	Escherichia coli	155	30.5%
2.	Klebsiella pneumoniae	125	24.5%
3.	Proteus species	84	16.4%
4.	Pseudomonas aeruginosa	47	9.2%
5.	Acinetobacter species	40	7.8%
6.	Enterobacter species	32	6.2%
7.	Citerobacter species	16	3.2%
8.	Morganella morganii	06	1.2%
9.	Serratia marcescens	05	1.0%
	Total	510	100%

Table-III

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	18 (20%)	12 (12%)	14 (14%)	15 (15%)	38(39%)

Discussion:

The rapidly rising rates of fluoroquinolone-resistant Esch.coli in many parts of the world has been found due to the reduced susceptibility to the quinolones. The Surveillance Network database (http: www.sur-net.world.com) shows resistance trends in blood-stream isolates from 250 U.S. hospitals as follows: Esch.coli, 1.8% in 1996 & 4.3% in 1999; Klebsiella spp., 7.1% in 1996 & 6.7% in 1999; Enterobacter spp., 6.6% in 1996 & 6.5% in 1999; & Proteus mirabilis, 5.7% in 1996 & 12.7% in 1999. High rates in Esch.coli may reflect contamination via the food chain; the Spanish study found quinolone-resistant Esch.coli & 90% of chicken feces & noted similar fecal carriage rates of resistant Esch.coli in children & adults. There is a small set of drugs commonly used to treat Ps.aeruginosa infection, including Ciprofloxacin, Tobramycin, Amikacin, Gentamicin, Ceftazidime, Piperacillin, Tazocin & Imipenem. While Ps.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, but Ps.aeruginosa rapidly acquires high level resistance rendering the drug important.

The resistance rates for Ciprofloxacin was 19% in our 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. 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 E, 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. Hence, it severely limits the already restricted treatment options. The finding was in accordance with the finding of a study which was conducted by Paterson *et al*. The high resistance pattern which was seen in our study was probably due to the inappropriate prescribing of antibiotics (sometimes without doing culture & sensitivity tests), lack of antibiotic policy & the poor

infection control strategies. But the antibiotic history could not be properly elicited from the patients in this study.

Ciprofloxacin remains a potent antibiotic; but the slow accumulation of resistant *Enterobacteriaceae* is disturbing, not least because resistance is a class effect, affecting all fluoroquinolones. 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 & animals, coupled with an emphasis on preventing patient-to-patient spread of resistant strains.

The antibiotic which showed maximum activity against most of the isolates was Imipenem & Tazocin. 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.

Conclusion:

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

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