ISSN: 2309-3234

TMSS MEDICAL COLLEGE JOURNAL

Volume 03, No. 1, July 2014

AN OFFICIAL PUBLICATION OF TMSS MEDICAL COLLEGE, BOGRA

TMSS MEDICAL COLLEGE JOURNAL
JULY 2014

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TMSS Medical College Journal

Vol. 3, No. 1, July 2014

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ORIGINAL ARTICLE

PREVALENCE OF CIPROFLOXACIN RESISTANCE AMONG GRAM-NEGATIVE BACILLI ISOLATED FROM URINARY TRACT INFECTION SPECIMENS AT A SPECIALIZED HOSPITAL IN RIYADH, SAUDI ARABIA

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ABSTRACT

Background: Antimicrobials of different structural classes including the fluoroquinolones have shown resistance in a multitude of bacterial species in the hospitals and in the community. Decreased susceptibility to fluoroquinolones arises mainly by single step mutations in the gyrA and 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 of resistance can generate therapeutic failure. In 1998, some mobile elements with a potential for the horizontal transfer of the quinolone resistance genes were described. The loci which are responsible for this plasmid-mediated quinolone resistance, which have been designated as qnr A, qnr B and qnr S, have been identified in the Enterobacteriaceae species. **Aim:** To evaluate the susceptibility pattern of the isolates to various antibiotics and to know the prevalence rate of Ciprofloxacin resistance in King Saud Medical Complex. **Materials and Methods:** A total of 510 gram-negative bacilli (GNB) were isolated from specimens of clinically diagnosed UTI patients over a period of six months (from January 2009 to June 2009) were subjected to antibiotic susceptibility testing. Isolates with resistance or with a decreased susceptibility to Ciprofloxacin (Zone of inhibition of bacterial growth around 20 mm of the E-strip) were then screened for their minimum inhibitory concentration (MIC) by using the E-strip 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. 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.

Key words: Gram-negative bacilli, MIC, Fluoroquinolone, Ciprofloxacin.

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 and occasionally for shigellosis, the fluoroquinolones have a broad range of therapeutic indications and are given as prophylaxis, e.g., in veterinary medicine fluoroquinolones are used as treatment and metaphylaxis but not as growth promoters. Early researchers thought that fluoroquinolones resistance was unlikely to evolve, largely because resistant Esch.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. Nevertheless multi-national quinolone resistance emerges in Staphylococci and Pseudomonads, which are inherently less susceptible than Esch.coli. More recently, fluoroquinolone resistance has emerged in Esch.coli and other Enterobactericeae, contingent on multiple mutations that diminish the affinity of its topisomerase II and IV targets in varying ways reduce permeability and up regulate efflux. Plasmid-mediated quinolone resistance has been reported but it is exceptional.

Ciprofloxacin is an antibiotic which is used in UTIs and active against gram-negative bacteria, which belongs to the fluoroquinolone class. Bacterial resistance is a growing therapeutic problem, both in the community and 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 and the par C genes, which encode the 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 and to know the prevalence rate of Ciprofloxacin resistance in our hospital.

MATERIAL AND METHODS

A total of 510 gram-negative bacilli were isolated from specimens of clinically diagnosed OPD and IPD UTI patients. The specimens were urine samples (mid-stream and morning sample) collected from urethral catheters and supra-pubic puncture channels. Specimens received in the Bacteriology Laboratory of King Saud Medical Complex over a period of six months (January 2009 to June 2009) were processed using different media like Sheep Blood Agar, MacConkey's Agarand Cystine Lactose Electrolyte Deficient (CLED) Agar. All isolates were identified by using standard biochemical kits, API 20E (Analytical Profile Index System and La Balme Les Grottes, France). Fully automated analyzers such as PHOENIX, MICROSCAN and VITEK II were also used for the identification and sensitivity pattern of the pathogens. Antibiotic sensitivity testing was performed mainly by using the fully automated analyzers (Phoenix, Microscan and Vitek II) and 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 and 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 and resistant. Isolates with resistance or decreased susceptibility to Ciprofloxacin (20mm) were subjected to further study. The study design and protocol was approved by Research and Ethics Committee of the institute (King Saud Medical Complex).

Resistance to Ciprofloxacin was confirmed by break point minimum inhibitory concentration (MIC in g/ml) by using E-test strips and also by the fully automated analyzers (Phoenix, Microscan and Vitek II). The isolates with MIC value 4 g/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%), and Serrtia marcescens (1.0%) as shown in Table-I.

Table-I: Total number of Gram-Negative Bacilli isolated from clinical specimens of UTIs (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%

Out of 510 gram-negative bacilli, (97=19%) isolates were resistant to Ciprofloxacin. High rates of resistance were

observed for Ampicillin and Cephalothin followed by Amoxicillin-Clavulanic Acid, Trimethoprim-Sulpha-methoxazole, while low levels of resistance were observed for Imipenem,

Table-II: Antibiotic susceptibility pattern of isolates to various antibiotics (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%)

Tazocin, Amikacin, Nitrofurantoin,Nalidixic Acid, Gentamicin and Norfloxacin, as shown in Table-II.

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 ‡32g/ml (Table-III)

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

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

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 and Proteus spp. followed by Esch.coli.

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 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. High rates in Esch.coli may reflect contamination via the food chain; the Spanish study found quinolone-resistant Esch.coli and 90% of chicken feces and noted similar fecal carriage rates of resistant Esch.coli in children and adults. There is a small set of drugs commonly used to treat Pseudomonas aeruginosa infection, including Ciprofloxacin, Tobramycin, Amikacin, Gentamicin, Ceftazidime, Piperacillin, Tazocin and Imipenem. While Pseudomonas 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 Pseudomonas aeruginosa rapidly acquires high level resistance rendering the drug important.

The resistance rate 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 and sensitivity tests), lack of antibiotic policy and 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 and 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 and 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 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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