Original article

Prevalence of Ciprofloxacin Resistance Among Gram-Negative Bacilli at a Specialist Hospital in Saudi Arabia

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Abstract:

Background: Resistance to antimicrobials of different structural classes including fluoroquinolones has arisen in a multitude of bacterial species both in the community and the hospitals. This may complicate the therapeutic management of infections. Decreased susceptibility to fluoroquinolones arises mainly by single-step mutations in the *gyrA* and *parC* genes, which encode the fluoroquinolones targets, the topoisomerase enzymes, conferring cross resistance to all fluoroquinolones. Accumulation of multiple mutations in several genes confers increasing level of resistance associated with clinical failure. However, even low level 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 qnrA, qnrB and qnrS, 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 our hospital. ***Materials & Methods:*** A total of 916 gram-negative bacilli (GNB) were isolated from different clinical specimens over a period of nine months, were subjected to antibiotic susceptibility testing. Isolates with resistance or with a decreased susceptibility to ciprofloxacin (£20 mm) were then screened for their minimum inhibitory concentration (MIC) by using the E-test. ***Results:*** Out of 916 GNB, 321 (35%) isolates were resistant to ciprofloxacin. The MIC of these isolates ranged from 4 to >32?g/ml. ***Conclusion:*** The resistance rate to ciprofloxacin was 35% in our study. Most of the ciprofloxacin resistant isolates were from urinary tract infections (UTI). 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 multidrug 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 and a need for the continuous evaluation of the commonly used antibiotics.

Key Words: Gram-negative bacilli, Fluoroquinolones, Ciprofloxacin, MIC

Introduction:

Fluoroquinolone antimicrobial drugs were a major therapeutic advance of the 1980s because they have 100-fold greater activity than their parent compound, nalidixic acid [1]. Unlike nalidixic acid, which is used only for urinary infections and occasionally shigellosis, the fluoroquinolones have a broad range of therapeutic indications and are given as prophylaxis, e.g., for in veterinary medicine fluoroquinolones are used as treatment and metaphylaxis but not as growth promoters. Early researchers thought that fluoroquinolone resistance was unlikely to evolve, largely because resistant *Escherichia coli* mutants are exceptionally difficult to select in vitro [2] and because plasmid-mediated quinolone resistance remained unknown even after 30 years of nalidixic acid usage. Nevertheless, mutational fluoroquinolone resistance emerged readily in staphylococci and pseudomonads, which are inherently less susceptible than *E. coli*. More recently, fluoroquinolone resistance has emerged in *E. coli* and other *Enterobacteriaceae*, contingent on multiple mutations that diminish the affinity of its topoisomerase II and IV targets in varying ways reduce permeability, and up regulate efflux[3]. Plasmid-mediated quinolone resistance has been reported, but it is exceptional[4].

Ciprofloxacin is a broad-spectrum antibiotic which is active against both gram-positive and gram-negative bacteria, which belongs to the fluoroquinolone class [5]. Bacterial resistance is a growing therapeutic problem, both in the community and the hospitals, involving all the antibiotics, which include fluoroquinolones. A decreased susceptibility to fluoro-

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quinolones arises mainly due to single-step mutations in the *gyrA* and the *parC* genes, which encode the fluoroquinolones targets, the topoisomerase enzymes[6]. In 1998, some mobile elements which were responsible for the horizontal transfer of the quinolone resistance genes were described[7,8]. This study was undertaken to evaluate the susceptibility of GNB to various antibiotics and to know the prevalence rate of ciprofloxacin resistance in our hospital.

Materials and Methods:

A total of 916 gram-negative bacilli were isolated from different clinical specimens i.e., urine, pus, sputum, blood etc, received in the Microbiology Laboratory over a period of nine months were subjected to the study. Specimens were processed using different media like MacConkey's agar, Cystein Lactose Electrolyte Deficient (CLED) Agar, Sheep Blood Agar and Chocolate Agar. All isolates were identified using standard biochemical tests[9]. In addition commercially available biochemical kits, API 20E (Analytic Profile Index system, La Balme les Grottes, France) were also used for the identification of enteric pathogens.

Antibiotic sensitivity testing was performed 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 was adjusted to the recommended turbidities for all species[10].

The antibiotics tested on each disc were Ampicillin 25 µg, Amoxicillin-Clavulanic Acid (20/10 µg), Trimethoprim-Sulphamethoxazole (1.25/23.75 µg), Cephalothin 30 µg, Cefuroxime 30 µg, Cefotaxime 30 µg, Ciprofloxacin 5 ?g, Norfloxacin 30 µg (for urinary isolates), Nalidixic Acid 30 µg (for urinary isolates), Nitrofurantoin 300 µg (for urinary isolates), Gentamicin 10 µg, Amikacin 30 µg and Imipenem 30 µg.

The Clinical Laboratory Standards Institute (CLSI) break points were used for interpretation of susceptibility patterns as sensitive or resistant[11]. Isolates with resistance or with decreased susceptibility to Ciprofloxacin (?20mm) were subjected to further study. This study design and protocol was approved by 'Research and Ethics Committee' of the institute.

E-Test

The resistance to ciprofloxacin was confirmed by breakpoint minimum inhibitory concentration (MIC in ?g/ml) by using E-test strips. The isolates with MIC value ?4 ?g/ml were defined as resistant isolates, as outlined by CLSI guidelines[11].

Results:*Escherichia coli* (29.4%) was the most predominant isolate which was found among the GNB, followed by *Klebsiella pneumoniae* (26.2%) *Pseudomonas aeruginosa* (25.0%) and *Proteus species* (12.9%) as shown in Table I.

Table I: Total number of Gram-negative Bacilli isolated from different clinical specimens (n=916)

S. No.	Organism	Total number isolated	Percentage (%)
1.	*Escherichia coli*	269	29.4%
2.	*Klebsiella pneumoniae*	240	26.2%
3.	*Pseudomonas aeruginosa*	229	25.0
4.	*Proteus species*	118	12.9%
5.	*Acinetobacter species*	49	5.3%
6.	*Citrobacter species*	11	1.2%
	Total	916	100.0%

Out of 916 gram-negative bacilli, 321 (35%) isolates were resistant to ciprofloxacin. High rates of resistance were observed for Ampicillin and Amoxicillin-Clavulanic Acid, followed by Cephalothin, Trimethoprim-Sulphamethoxazole, and cefotaxime, while low levels of resistance were observed for nitrofurantoin, nalidixic acid, amikacin and norfloxacin, as shown in Table II.

Table II: Antibiotic Susceptibility pattern of the isolates to various antibiotics (n=916)

S. No.	Antibiotics	Total no of Sensitive isolates (%)	Total no of Resistant isolates (%)
1.	Ampicillin	192 (21%)	724 (79%)
2.	Amoxicillin-Clavulanic Acid	229 (25%)	687 (75%)
3.	Trimethoprim-Sulphamethoxazole	367 (40%)	549 (60%)
4.	Cephalothin	357 (39%)	559 (61%)
5.	Cefuroxime	679 (74%)	237 (26%)
6.	Cefotaxime	375 (41%)	541 (59%)

7.	Ciprofloxacin	595 (65%)	321 (35%)
8.	Norfloxacin(for urinary isolates=321 GNB)	247 (77%)	74 (23%)
9.	Nalidixic Acid(for urinary isolates=321 GNB)	254 (79%)	67 (21%)
10.	Nitrofurantoin (for urinary isolates=321 GNB)	257 (80%)	64 (20%)
11.	Gentamicin	632 (69%)	284 (31%)
12.	Amikacin	742 (81%)	174 (19%)
13.	Imipenem	879 (96%)	37 (4%)

The lowest level of resistance was observed for imipenem (4%). The resistance rate for ciprofloxacin was 35%. The MIC of ciprofloxacin for these isolates ranged from 4 to >32 ?g/ml (Table III).

Table III: MIC values of the resistant Gram Negative Bacilli to Ciprofloxacin (n=321)

Ciprofloxacin MIC values	4µg/ ml	8µg/ ml	16µg/ ml	32µg/ ml	>32µg/ ml
Total No. of isolates	77 (24%)	42 (13%)	39 (12%)	51 (16%)	112 (35%)

The isolated bacteria showed wide differences in their susceptibility to ciprofloxacin. A high rate of resistance to ciprofloxacin was observed among Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter sps., and Proteus sps. followed by E. coli.

Discussion:

Evolution of reduced susceptibility to the quinolones is causing concern following rapidly rising rates of fluoroquinolone- resistant E. coli in many parts of the world[12]. The Surveillance Network database (http://www.mrlworld.com) shows resistance trends (with intermediate counted as resistant) in bloodstream isolates from 250 U.S. hospitals as follows: *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 *P. mirabilis*, 5.7% in 1996 and 12.7% in 1999. Much higher rates are reported from Barcelona, Spain, where 17% of *E. coli* isolates from community infections were ciprofloxacin resistant[13], and India, where up to 50% of hospital *E. coli* are reported resistant[14]. High rates in *E. coli* may reflect contamination via the food chain: the Spanish study found quinolone-resistant *E. coli* in 90% of chicken feces and noted similar fecal carriage rates of resistant *E. coli* in children and adults. There is a small set of drugs commonly used to treat *P. aeruginosa* infection, including ciprofloxacin, tobramycin, gentamicin, ceftazidime, and imipenem. 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, but *P. aeruginosa* rapidly acquires high-level resistance, rendering the drug impotent. In clinical isolates, approximately 30% of strains now present high-level ciprofloxacin resistance[15].

The resistance rate for ciprofloxacin was 35% in our study. Most of the ciprofloxacin resistant isolates were obtained from UTI samples. This may be because 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[16]. This emphasises 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 and imipenem. This was in agreement with the finding of a study reported by Astal ZE, 2005[17].

These data suggest that nitrofurantoin can still be successfully used in the treatment of UTI. The ciprofloxacin resistance was also closely associated with multi-drug resistance, thus making the treatment options limited[18]. Ciprofloxacin resistance can be used as a general surrogate marker of multi-drug resistance. Hence, it severely limits the already restricted treatment options. This finding was in accordance with the finding of a study which was conducted by Paterson *et al*[19]. The high resistance pattern which was seen in our study was probably due to the inappropriate prescribing of antibiotics, 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 inhibiting the efflux pumps that contribute to the resistance[20],

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. 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 carbapenem resistance.

Conclusion:

The considerably high MIC values for ciprofloxacin in this study reflect the 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 prescription 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.

References:

1. Bauernfeind A, Petermuller C. In vitro activity of ciprofloxacin, norfloxacin and nalidixic acid. *Eur J Clin Microbiol* 1983; **2**:111–5. http://dx.doi.org/10.1007/BF02001575 PMid:6222896

2. Smith JT. The mode of action of 4-quinolones and possible mechanisms of resistance. *J Antimicrob Chemother* 1986; **18**:21-9. PMid:3542946

3. Everett MJ, Jin YF, Ricci V, Piddock LJ. Contributions of individual mechanisms to fluoroquinolone resistance in 36 Escherichia coli strains isolated from humans and animals. *Antimicrob Agents Chemother* 1996; **40**:2380–6. PMid:8891148 PMCid:163538

4. Martinez-Martinez L, Pascual A, Jacoby GA. Quinolone resistance from a transferable plasmid. *Lancet* 1998; **351**:797–9. http://dx.doi.org/10.1016/S0140 6736(97)07322-4

5. Drlica K, Zhao XK. DNA gyrase, topoisomerase IV and 4-quinolone. *Microbiol Mol Biol Rev* 1997; **61**(3): 377-92. PMid:9293187 PMCid:232616

6. Hooper DC. The emerging mechanisms of fluorquinolone resistance. *Emerg Infect Dis* 2001; **7:**337-41. http://dx.doi.org/10. 3201/eid0702.010239PMid:11294736 PMCid:2631735

7. Martinez-Martinez L, Pascual A, Jacoby GA. Quinolone resistance from a transferable plasmid. *Lancet* 1998; **351**:797-9.http://dx.doi.org/10.1016/S0140 6736(97)07322-4

8. Nordmann P, Poirel L. The emergence of plasmid-mediated resistance to quinolones in Enterobacteriaceae. *J Anti-microb Chemother* 2005; **56**:463-9. http://dx.doi.org/10.1093/jac/dki245PMid:16020539

9. Mackie and McCartney. *Textbook of Practical Medical Microbiology. 14th edition*; 153, 416.

10. Ericsson HM, Sherris JS. Antibiotic sensitivity testing. Report of an International Collaborative Study. *Acta Pathologica Scandinavica* 1971; **Section B Suppl**: 1-89.

11. CLSI. *Performance standards for antimicrobial susceptibility testing. 2009*; **M100-S19 CLSI**, Wayne, PA.

12. Livermore DM: Has the era of untreatable infections arrived? *J Antimicrob Chemother* 2009; **64** (1):29-36.http://dx.doi.org/10.1093/jac/dkp255 PMid:19675016

13. Zinner SH. Changing epidemiology of infections in patients with neutropenia and cancer: emphasis on gram-positive and resistant bacteria. *Clin Infect Dis* 1999; **29**:490–4. http://dx.doi.org/10.1086/598620PMid:10530434

14. Garau J, Xercavins M, Rodriguez-Carballeira M, Gomez-Vera JR, Coll I, Vidal D, Emergence and dissemination of quinolone-resistant

Escherichia coli in the community. *Antimicrob Agents Chemother* 1999; **43**:2736–41. PMid:10543756 PMCid:89552

15. Manno, G., M. Cruciani, L. Romano, S. Scapolan, M. Mentasti, R. Lorini, and L. Minicucci. Antimicrobial use and Pseudomonas aeruginosa susceptibility profile in a cystic fibrosis centre. Int. J. Antimicrob. *Agents* 2005; **25**:193–197.http://dx.doi.org/10.1016/j.ijantimicag.2004.11.009PMid:15737511

16. Schaeffer AJ. The expanding role of fluoroquinolones. *Am J Med 2002*; **113**(suppl 1A):45S–54S.http://dx.doi.org/10.1016/S0002 9343(02)01059-8

17. Astal ZE. The increasing ciprofloxacin resistance among prevalent urinary tract bacterial isolates in Gaza Strip. *Singapore Med J* 2005; **46**(9):457-60.PMid:16123829

18. Kang CI, Kim SH, Kim DM, et al. The risk factors for ciprofloxacin resistance in bloodstream infections which were caused due to extended-spectrum ?-lactamase producing Escherichia coli and Klebsiella pneumoniae. *Microb Drug Resist* 2004; **10**: 71-76. http://dx.doi.org/10.1089/107662904323047835 PMid:15140397

19. Paterson DL, Mulazimoglu L, Casellas JM, Ko WC, Goossens H, Von Gottberg A, Mohapatra S, Trenholme GM, Klugman KP, McCormack JG, Yu VL. Epidemiology of ciprofloxacin resistance and its relationship to extended spectrum ?-lactamase production in Klebsiella pneumoniae isolates, which caused bacteremia. *Clin Infect Dis* 2000; **30**(3): 473-78. http://dx.doi.org/10.1086/313719PMid:10722430

20. Nema S, Premchandani P, Asolkar MV, Chitnis DS. Emerging bacterial drug resistance in hospital practice. *Indian J Med Sci* 1997; **51**:275–80. PMid:9491681

21. MM Rahman. Molecular methods in medical microbiology: Current and future trends. *Bangladesh Journal of Medical Science* 2011; **10**(3): 141-147. DOI: http://dx.doi.org/10.3329/bjms.v10i3.8355