Comparison of minimum inhibitory concentration values for fluoroquinolones against *Escherichia coli* causing urinary tract infection in both hospitalized patients and outpatients

Mohamed Tayfour, *PhD,* ***A. Yuce,*** *MD,* ***N. Yulug,*** *MD.*

ABSTRACT

Objective: To determine the resistance among *Escherichia coli* isolates causing urinary tract infections in hospitalized patients and outpatients.

Methods: This study was carried out in the Department of Microbiology, Dokuz Eylul University, Medical Faculty Hospital, Inciralti, Ismir, Turkey, from February 1997 through to June 1998. A total of 300 *Escherichia coli* strains were isolated from urine specimens of 111 hospitalized and 189 elderly outpatients (more than 20 years of age). We determined the minimum inhibitory concentrations of the test drugs nalidixic acid, pefloxacin, ofloxacin, norfloxacin and ciprofloxacin by the microdilution method, recommended by the National Committee for Clinical Laboratory Standards.

Results: Minimum inhibitory concentrations$_{50}$ and minimum inhibitory concentrations$_{90}$ values of strains tested against fluoroquinolones, pefloxacin and nalidixic acid were the same for strains isolated from hospitalized and outpatients (0.125μg/ml outpatients (0.03μg/ml). Twenty-six (9%) of 300 *Escherichia coli* strains were resistant to all drugs used. Twenty (77%) of these 26 strains were isolated from hospitalized patients. We found that the resistance to nalidixic acid is much higher than other fluoroquinolones. At the same time, the resistance in the strains that were isolated from hospitalized patients is again higher than outpatient strains (46%).

Conclusion: Resistance among *Escherichia coli* isolates from patients to quinolones used in the treatment of urinary tract infections was rare during this period. Our study, like many other reports showed the increased resistance to fluoroquinolones for clinical isolates. However the appearance of multi resistant clones and the elevated prevalence of quinolones resistance in the hospital studied are warning signals for an increase in resistant strains as seen in many other countries. Therefore, it is important for physicians to use fluoroquinolones carefully so as to prevent, or delay, the emergence of resistant strains.

Keywords: *Escherichia coli,* minimum inhibitory concentration, fluoroquinolone, urinary tract infection.

Saudi Med J 2001; Vol. 22 (10): 848-851

Quinolones are bactericidal antibiotics that inhibit bacterial deoxyribonucleic acid (DNA) replication. The quinolone nalidixic acid (NA), was developed initially as a urinary antiseptic. A new

From the Department of Microbiology, (Tayfour) King Fahad Hospital, Al-Baha, Kingdom of Saudi Arabia, and the Department of Microbiology, (Yuce Yulug), Dokuz Eylul University, Medical Faculty Hospital, Inciralti, Ismir, Turkey.

Received 8th April 2000. Accepted for publication in final form 3rd April 2001.

Address correspondence and reprint request to: Dr. Mohamed Tayfour, Department of Microbiology, King Fahad Hospital, PO Box 204, Al-Baha, Kingdom of Saudi Arabia. Tel +966 (7) 7254000. Fax +966 (7) 7251732.

class of quinolones, also known as fluoroquinolones, have been synthesized by modifying the original 2 ring quinolon nucleus with different side chain substitution.[1] Fluoroquinolones are bactericidal antibiotics that bind to the b-subunit of DNA gyrase, an essential enzyme for DNA replication.[2] Deoxyribonucleic acid gyrase, an A_2B_2 tetrameric enzyme and the target of the quinolones, is a Type II topoisomerase that is essential for replication and gene expression and is also involved in recombination and conjugation.[3-6] Quinolone antibiotics are highly active against gram negative bacilli, gram positive species and other micro organisms like *Legionella, Mycoplasma, Chlamydia, Plasmodium* and intracellular bacterial species such as *Mycobacteria* and *Rickettsii*.[3,7-14] Since 1962, when the first quinolone NA was used in treatment and since 1984 when the first fluoroquinolone norfloxacin (NOR) was marketed in Europe, there has been a marked increase in the usage of this class of drugs. The widespread use of fluoroquinolones has increased resistance to these drugs in clinical isolates of many bacteria such as *E.coli*.[15] Different mechanisms of resistance to fluoroquinolones are known: 1. Decrease in membrane permeability or reduction of the quinolone accumulation and 2. Alteration of the molecular target of quinolone action DNA gyrase, and 3. Increased efflux of quinolones.[3,5,15-21] *Escherichia coli* is the most common agent in urinary tract infections (UTIs) especially in hospitalized patients.[22-24] In this study, in vitro activities of fluoroquinolones were investigated in 300 *E.coli* strains isolated from UTI cases both in hospitalized patiets and outpatients.

Methods.

Microorganisms. A total of 300 *E.coli* strains were isolated from urine specimens of 111 hospitalized patients and 189 outpatients submitted to our Microbiology Department, Dokuz Eylul University, Medical Faculty Hospital, Inciralti, Ismir, Turkey, from February 1997 to June 1998. The isolated strains were collected randomly from both male and female elderly patients. The identification of all isolates was confirmed by using standard biochemical methods.[1,25] *Escherichia coli* American Type Culture Collection (ATCC) 25922 was used as a control strain

Drugs. All drugs were purchased from the appropriate pharmaceutical companies and were in powder form. Nalidixic acid was dissolved with sodium hydroxide (NaOH) and than diluted with Müller-Hinton (MH) broth. Other fluoroquinolones; pefloxacin (PEF), ofloxacin (OFX), NOR and ciprofloxacin (CIP) were dissolved in distilled water and diluted with MH broth as described previously.[26]

Inoculum. A standard inoculum of the microorganism used was 10^5 cfu/ml as described previously.[26]

Table 1 - Interpretation of sensitivity tests according to minimum inhibitory concentration (MIC) values (mg/I).

Drugs	Sensitive	Resistant	Intermediate
Pefloxacin	4 mg/I <	2 mg/I <	1 mg/I >
Ofloxacin	8 mg/I <	4 mg/I <	2 mg/I >
Norfloxacin	16 mg/I <	8 mg/I <	4 mg/I >
Ciprofloxacin	4 mg/I <	2 mg/I <	1 mg/I >
Nalidixic acid	32 mg/I <	- mg/I <	16 mg/I >

Table 2 - Minimal inhibitory concentration 50 (MIC) and minimal inhibitory concentration 90 (MIC) values of *Escherichia coli* strains against fluoroquinolones and nalidixic acid.

Strains	PEF MIC_{50} MIC_{90} μg/ml	OFX MIC_{50} MIC_{90} μg/ml	NOR MIC_{50} MIC_{90} μg/ml	CIP MIC_{50} MIC_{90} μg/ml	NA MIC_{50} MIC_{90} μg/ml
Hospitalized patient strains n=111	0.125/64	0.125/32	0.06/32	0.03/16	16/128
Oupatient strains n=189	0.50/1	0.125/1	0.125/1	0.03/0.125	8/64
PEF=pefloxacin, OFX=ofloxacin, NOR=norfloxacin, CIP=ciprofloxacin, NA=nalidixic acid, n=number					

Minimum inhibitory concentrations determination. We determined the MICs of the test drug by the microdilution method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS)[26] and read them after the 18 hours of incubation at 35°c. Minimum inhibitory $concentration_{50}$ and MIC_{90} values were detected according to the results of the microdilution tests and their sensitivity against fluoroquinolones and NA were interpreted according to the values in Table 1.[26]

Results.

Our MIC_{50} and MIC_{90} values of strains tested against fluoroquinolones and NA were shown in Table 2. The MIC_{50} values for OFX and CIP were the same for strains isolated from hospitalized (0.125 μg/ml) and outpatients (0.03 μg/ml). The resistance rates to fluoroquinolones according to their break points were shown in Table 3. Break points: PEF=4μg/ml, OFX=2μg/ml, NOR=4μg/ml, CIP=1μg/ml and NA=6μg/ml.[26] Twenty-six (9%) of 300 *E.coli* strains were resistant to all drugs used.

Table 3 - The resistance rates to fluoroquinolones according to their break points.

Strains	PEF n %	OFX n %	NOR n %	CIP n %	NA n %
Hospitalized patient strains n=111	17 (15)	23 (21)	23 (21)	20 (18)	51 (46)
Outpatient strains n=189	7 (4)	10 (5)	7 (4)	6 (3)	39 (21)

PEF=pefloxacin, OFX=ofloxacin, NOR=norfloxacin, CIP=ciprofloxacin, NA=nalidixic acid, n=number

Twenty (77%) of these 26 strains were isolated from hospitalized patients. We observed that the resistance to NA is much higher than other fluoroquinolones. The resistance rate in hospitalized patient strains was 46% and in outpatient strains 21% as shown in Table 3.

Discussion. Fluoroquinolones are commonly used for the treatment of outpatients and nosocomial infections caused by gram-negative bacilli and gram-positive species.[3,11,15,27-29] The size of the antibiotic market in developing countries is double than that seen in a developed country.[29] In developing countries the resistance to quinolones has been increasing yearly.[30] Our study showed that the resistance for quinolones to *E.coli* strains isolated from urine specimens is higher for hospitalized patients as compared to outpatients. Twenty-six (9%) of 300 strains were resistant to all used quinolones, and 20 (77%) of these strains were isolated from hospitalized patients (n=111). On the other hand, we observed that the resistance rate to NA in *E.coli* strains isolated both in hospitalized patients and outpatients was very high. Like our study, many other reports have shown increased resistance to fluoroquinolones by various clinical isolates.[10,11,30,31] Özkan et al[10] found the resistance rate to quinolones OFX as 8%, CIP as 7%, NOR as 8%, and PEF as 10%. Çöplü et al [31] studied 100 *E.coli* strains isolated from urine specimens of hospitalized patients and found 13-18% resistance rate to various quinolones. Aydn et al[11] found resistance rates of *E.coli* strains to various quinolones as following: NA 9-15%, NOR 0-6%, OFX 0-15%, PEF 12-17% and CIP 4-14%. Perez-Trallero et al[30] found in a prospective study between 1989 and 1992 that uropathogen *E.coli* resistance to CIP increased from 1%-7%. It is known that antibiotic resistance differs depending on the country, region, the patients from whom they are isolated and the antibiotic policy of that place. Widespread usage of fluoroquinolones without any national antibiotic usage program in Turkey, the clinical diagnosis of patients, a history of patients like previous infection or immunosupression may have influenced our finding of a high rate of resistance in hospitalized patient strains. Therefore, it is important for physicians to use fluoroquinolones carefully so as to prevent or delay the emergence of resistant strains.

References

1. Yao JDC, Moellering RJ Jr. Antibacterial agents. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH editors. Manual of Clinical Microbiology. 6th ed. Washington DC: ASM; 1995. p. 1281-1307.
2. Slayers AA, Whitt DD. Bacterial pathogenesis: A molecular Approach. Washington DC (NY): American Society for Microbiology; 1994. p. 101.
3. Cambau E, Bordon F, Collatz E, Gutmann L. Novel gyr a point mutation in a strain of E.coli resistant to Fluoroquinolones but not to Nalidixic acid. Antimicrob Agents Chemother 1993; 36: 1247-1252.
4. Cozzarelli NR. DNA gyrase and the supercoiling of DNA. Science 1980; 207: 953-960.
5. Wiedemann B, Heisig P. Mechanisms of quinolone resistance. Infection 1994; 2: 73-79.
6. Palumbo M, Gatto B, Zagatto G, Palu G. On the mechanism of action of quinolone drugs. Trends Microbiol 1993; 1: 232-235.
7. McClean KM, Hitchman D, Stephen DS. Norfloxacin is inferior to chloroquine for falciparum malaria in northwestern Zambia. A comparative clinical trial. J Infect Dis 1992; 165: 904-907.
8. Tomioka H, Saito H, Sato K. Comparative antimycobacterial activities of the newly synthesized quinolone AM-1155, sparofloxacin and ofloxacin. Antimicrob Agents Chemother 1993; 36: 1259-1263.
9. Christopher EF, Wilcox CM, Cello JP, Margaretten W, Lee B, Sachdeeva M et al. Ciprofloxacin in the treatment of Helicobacter pylori in-patients with gastritis and peptic ulcer. J Infect Dis 1990; 162: 998-999.
10. Özkan F, Ulusoy S, Yüce K. The effective of Ofloxacin, ciprofloxacin, norfloxacin and pefloxacin to Escherichia coli in vitro. Ankem 1994; 8: 69-72.
11. Ayd in MD, Büyükbaba O ve Ang O. The Susceptibility of quinolones against Enterobacteriaceae that coast UTI. Ankem 1996; 8: 19-23.
12. Gohara Y, Arai S, Akashi A, Alrij BT. In vitro and in vivo activities of Q-35, a new fluoroquinolone against Mycoplasma pneumoniae. Antimicrob Agents Chemother 1993; 37: 1826-1830.
13. Carbon C. Quinolones in the treatment of lower respiratory tract infections in adult patients. Drugs 1993; 45 (Suppl 3): 91-97.
14. Just PM. Overview of fluoroquinolone antibiotics. Pharmacotherapy 1993; 13: 45-175.
15. Kresken M, Hafner D, Mittermayer H, Verbist L, Bergogne-Berezin E, Giamarellou H et al. Prevalence of fluoroquinolone resistance in Europe. Infection 1994; 22 (Suppl 2): 90-98.
16. Alarcon T, Pita J, Lopez BM, Piddik LJ. High-level quinolone resistance amongst clinical isolates of E.coli and Klebsiella pneumoniae from Spain. J Antimicrob Chemother 1993; 32: 605-609.
17. Chen YC, Chang SC, Lif HSN, Hsieh WC, Luk KT. In vitro antimicrobial activity of fluoroquinolones against clinical isolates obtained in 1989 and 1990. J Formos Med Assoc 1993; 92: 1040-1048.

18. Thornsberry C. Susceptibility of clinical bacterial isolates to ciprofloxacin in the United States. Infection 1994; 22: 2580-2589.
19. Dalhoff A, Bayer AG. Quinolone resistance in Pseudomonas aeruginosa and Staphylococcus aureus. Development during therapy and clinical significance. Infection 1994; 22 (Suppl 2): 111-121.
20. Naber KG, Witte W, Bauernfeind A, Wiedemann B, Wagenlehner F, Klare I et al. Clinical significance and spread of fluoroquinolone resistant uropathogenes in hospitalized uroligical patients. Infection 1994; 22 (Suppl 2): 122-1217.
21. Acar JF, Goldstein FW. Trend in bacterial resistance to fluoroquinolones. Clin Infect Dis 1997; 24 (Suppl 1): 67-73.
22. Unal S, Akali HE. Uriner sistem infeksiyonlari. In: Kanra G, Akali HE, editors. Infeksiyon Hastal iklari: Akut bakteriyel Infeksiyonlara Yaklasi M. 2. bask. Ankara (Turkey): Feryyat matbaasi. 1993. p. 167-189.
23. Mandell IG, Douglas RG, Bennett JE. Principles and practice of infection diseases. 4th ed. New York (NY): Churchill Livingston, 4th ed. 1995.
24. Korten V. Hastane infeksiyonlar ini n epidemiyolojisi ve genel risk faktorleri. In: Akali E, editors. Hastane infeksiyonlari . 1bask. Ankara (Turkey): Feryyat matbaasi. 1993. p. 34-44.
25. Finegold SM, Baron EJ. In: Manning S (editor). Bailey and Scott's Diagnostic Microbiology. 7th ed. St Louis, Baltimore, Philadelphia, Toronto: The CV Mosby Company; 1990. p. 100-126.
26. NCCLS method for dilution antimicrobial susceptibility tests for bacteria that growth aerobically. Approved standard M7-A4. Villanova 1997.
27. Richard P, Delangle MH, Merrien D, Barille S, Reynaud A, Minozzi C et al. Fluoroquinolone use and fluoroquinolone resistance. Is there an association? Clin Infect Dis 1994; 19: 54-59.
28. Acar JF, O'Brien JF, Goldestein FW, Jones RN. The epidemiology of bacterial resistance to quinolones. Drugs 1993; 45 (Suppl 3): 24-25.
29. Rodriguez NE, Martin OR, Esparza AS. The use of quinolones in developing countries. Drugs 1993; 45 (Suppl 3): 42-45.
30. Perez TE, Urbieta M, Jimenez D, Garcin AJM, Cilla G. Ten-year survey of quinolone resistance in E. coli causing urinary tract infections, Eur J Clin Microbiol Inf Dis 1993; 12: 349-351.
31. N Coplu, P Zarakolu, G Gursoy, Guvener E. Uriner sistem infeksiyonlarindan izole edilen E.coli suslari icin kinolonlarin MIK degerleri. Ankem 1994; 8: 19-22.