In vitro Activity of Fluoroquinolones against Common Respiratory Pathogens

S Aydemir[1], A Tunger[1], F Cilli[1]

ABSTRACT

The treatment of respiratory infections is often empiric, necessitating the use of agents with a broad range of antimicrobial activity. The fluoroquinolones, having activity against common respiratory pathogens, fit this description. New fluoroquinolones have been developed in an attempt to improve the in vitro activity against a wide variety of respiratory tract pathogens. The objective of the study is to compare in vitro *activity of newest fluoroquinolones, gatifloxacin and moxifloxacin, with levofloxacin and ciprofloxacin using three major respiratory pathogens,* Streptococcus pneumoniae, Haemophilus influenzae and Moraxella catarrhalis. *Minimum inhibitory concentrations (MICs) of four fluoroquinolones were tested against 93* S pneumoniae, *62* H influenzae *and 60* M catarrhalis, *ie 215 isolates by the E-test method. National Committee for Clinical Laboratory Standards (NCCLS)-approved interpretive criteria were used throughout. All isolates were susceptible to the tested fluoro-quinolones. Ninety per cent of* S pneumoniae *strains were inhibited by ciprofloxacin at concentrations of 2 mg/L. The gatifloxacin and moxifloxacin MICs were lower than the ciprofloxacin and levofloxacin MICs against* S pneumoniae. *In contrast to* S pneumoniae, in vitro *activities of gatifloxacin and moxifloxacin offered no apparent advantages over ciprofloxacin and levofloxacin for* H influenzae and M catarrhalis.

La Actividad *in Vitro* de las Fluorquinolonas Contra los Patógenos Respiratorios

S Aydemir[1], A Tunger[1], F Cilli[1]

RESUMEN

La terapia de las infecciones respiratorias es a menudo empírica, y exige por ende el uso de agentes con un amplio espectro de actividad antimicrobiana. Por su actividad contra los patógenos respiratorios comunes, las fluorquinolonas se ajustan a esta descripción. Nuevas fluorquinolonas han sido desarrolladas, en un intento por mejorar la actividad in vitro contra una variedad de patógenos de las vías respiratorias. El objetivo de este estudio es comparar la actividad in vitro *de las fluorquinolonas más recientes – la gatifloxacina y la moxifloxacina – con la levofloxacina y la ciprofloxacina, usando tres de los más importantes patógenos respiratorios: Streptococcus pneumoniae,* Haemophilus influenzae *y* Moraxella catarrhalis. *Las concentraciones inhibitorias mínimas (CIMs) de las cuatro fluorquinolonas fueron sometidas a prueba contra 93* S pneumoniae, *62* H influenzae *y 60* M catarrhalis, *para un total de 215 aislados mediante el método de E-test. En todos los casos se aplicaron criterios interpretativos aprobados por el Comité Nacional para Normas del Laboratorio Clínico (NCCLS). Todos los aislados resultaron sensibles a las fluorquinolonas ensayadas. El noventa por ciento de las cepas de* S pneumoniae *fueron inhibidas por la ciprofloxacina a concentrationes of 2 mg/L. Las CIMs de la gatifloxacina y la moxifloxacina fueron más bajas que las CIMs de la ciprofloxacina y la levofloxacina contra* S pneumoniae. En contraste *con* S pneumoniae, *la actividad in vitro de la gatifloxacina y la moxifloxacina no ofrecieron ventajas aparentes por encima de la ciprofloxacina y la levofloxacina frente a* H influenzae *y* M catarrhalis.

West Indian Med J 2006; 55 (1): 9

From: Ege University, Medical Faculty, Department of Microbiology and Clinical Microbiology[1], Izmir, Turkey

Correspondence: Dr S Aydemir, Ege University Medical Faculty, Department of Microbiology and Clinical Microbiology, Bornova, 35100, Izmir, Turkey. Fax: +90-232-324-5914, e-mail: sohret@med.ege.edu.tr.

INTRODUCTION

Antibiotic resistance among respiratory tract pathogens is increasing worldwide. Beta-lactam-resistant strains of the three most common pathogens, *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis*, are

being isolated with increasing frequency in many countries, as well as macrolide- and fluoroquinolone-resistant strains of *S pneumoniae* (1–5). Acute respiratory infections represent a major public health challenge for clinicians, therefore there is a need for new antimicrobials with activity against these micro-organisms. The new fluoroquinolones gatifloxacin and moxifloxacin have been developed in an attempt to improve this situation and physicians have turned to fluoroquinolones for empirical treatment of respiratory infections, including many due to pneumococci (6).

Since the introduction of nalidixic acid, the first quinolone, in 1962, structural modifications have resulted in the production of numerous agents in second-, third-, and fourth-generation fluoroquinolones. In December 1999, the US Food and Drug Administration (FDA) approved gatifloxacin and moxifloxacin, which show excellent *in vitro* activity against a wide variety of respiratory tract pathogens. These agents may be administered as oral and/or intravenous formulations with excellent bioavailability. The pharmacodynamics of these new fluoroquinolones are more favourable than those of levofloxacin or ciprofloxacin for S *pneumoniae*. These agents are approved for the treatment of acute exacerbation of chronic bronchitis, community-acquired pneumonia and sinusitis (7).

In this *in vitro* study, minimum inhibitory concentrations (MICs) were determined for major respiratory pathogens to four fluoroquinolones and were compared to determine whether the new generation fluoroquinolones, gatifloxacin and moxifloxacin offered any advantages over the levofloxacin and ciprofloxacin.

MATERIALS AND METHODS

During January to December 2003, 93 isolates of *S pneumoniae,* 62 isolates of *H influenzae*, and 60 isolates of *M catarrhalis* were collected from patients treated at the Hospital of Ege University Medical Faculty. A total of 215 isolates was obtained with 175 being from sputum, 12 from broncho-alveolar lavage and 28 from deep tracheal aspirate. Only one isolate per patient was included.

All isolates were identified according to the standard microbiology criteria by using conventional methods as well as commercial identification systems, API NH (bioMérieux, France) for *M catarrhalis* and *H influenzae.*

MIC values of gatifloxacin, moxifloxacin, levofloxacin and ciprofloxacin were determined by the E-test (AB-Biodisk, Sweden) method on Mueller-Hinton agar (Oxoid) with 5% sheep blood for *S pneumoniae*, on Mueller-Hinton agar (Oxoid) for *M catarrhalis*, and on Haemophilus Test Medium (Oxoid) for *H influenzae* strains. Agar plates used for susceptibility testing were inoculated in a suspension of organisms having the opacity of 0.5 McFarland turbidity standard. Inoculated plates were allowed to dry before E-test strips containing gatifloxacin, moxifloxacin, levofloxacin and ciprofloxacin were applied to the surface of the agar. After incubation for 22 to 24 hours at 37°C in an atmosphere of five per cent CO_2, the MICs were read directly from the intersection of the inhibition ellipse with the test strip MIC scale. MIC results were evaluated according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines except in the case of ciprofloxacin for pneumococci where ciprofloxacin resistance was defined as an MIC $ 4 mg/L in keeping with the definition that has been used before in the literature (8, 9). For *M catarrhalis* strains, breakpoints for *H influenzae* were applied (10).

The control strains included were *S pneumoniae* ATCC 29213 and *H influenzae* ATCC 49247 on each set of testing.

RESULTS

All the *S pneumoniae* strains taken in this study had MIC values lower than 4 mg/L to ciprofloxacin and MIC_{50} and MIC_{90} of ciprofloxacin were 1 mg/L and 2 mg/L, respectively (range 0.25-3 mg/L). All the *S pneumoniae* isolates were susceptible to levofloxacin, gatifloxacin and moxifloxacin having MIC_{90} as 2 mg/L, 0.50 mg/L and 0.38 mg/L, respectively.

No *H influenzae* isolates tested had an MIC > 0.125 mg/L to any of the fluoroquinolones. MIC_{90} values for all fluoroquinolones tested were 0.032-0.125 mg/L, several-fold lower than the susceptibility breakpoints defined by NCCLS. All *M catarrhalis* isolates were susceptible to all four quinolones tested. They inhibited all isolates at concentrations between 0.008 and 0.125 mg/L.

The MIC ranges, MIC_{50} values and MIC_{90} values for all the quinolones tested are shown in the Table.

Table: Activity of four fluoroquinolones against *S pneumoniae, H influenzae and M catarrhalis* isolates

Bacteria/Antibiotics	Range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)
S pneumoniae (n = 93)			
Ciprofloxacin	0.25 – 3	1	2
Levofloxacin	0.38 – 2	1	2
Gatifloxacin	0.094 –1	0.38	0.50
Moxifloxacin	0.125 – 0.50	0.25	0.38
H influenzae (n = 62)			
Ciprofloxacin	0.008 – 0.125	0.016	0.032
Levofloxacin	0.023 – 0.125	0.032	0.064
Gatifloxacin	0.008 – 0.125	0.023	0.047
Moxifloxacin	0.032 – 0.125	0.064	0.125
M catarrhalis (n = 60)			
Ciprofloxacin	0.008 – 0.064	0.016	0.032
Levofloxacin	0.016 – 0.094	0.032	0.064
Gatifloxacin	0.016 – 0.125	0.047	0.064
Moxifloxacin	0.016 – 0.125	0.064	0.094

DISCUSSION

Respiratory infections are a common cause of morbidity, pneumonia being the most common infectious cause of death in a number of developed countries (11). Community acquired pneumonia is frequently diagnosed and treated on clinical and radiological findings only. For various reasons,

microbiological data are not always available (geographical problems, patients who do not produce sputum *etc*) and, even when the most extensive microbiological tests are carried out, in more than 50% of cases they do not give any useful results (12). Thus, empirical treatment is frequently necessary. When prescribing an empirical treatment antibiotics effective against all the likely pathogens are needed (13). In this study, major respiratory pathogens *S pneumoniae, H influenzae* and *M catarrhalis* were examined.

Several surveillance studies have shown that antimicrobial resistance of these three major respiratory pathogens is an increasing problem throughout the world. This makes the fluoroquinolones an attractive alternative. In our region, authors reported among *S pneumoniae* strains 29% intermediate- and 3% high-level penicillin resistance, trimethoprim-sulfamethoxazole (TMP-SMX) resistance rate is even higher, about 64%. The rate of resistance to clarithromycin is higher (32.7%) in strains showing intermediate-level penicillin resistance than penicillin-susceptible strains (5.8%). Among *H influenzae* and *M catarrhalis* strains, the rate of beta-lactamase production is 3.8% and 44.4%, respectively. TMP-SMX resistance of *H influenzae* is as high as 31.6% compared to the macrolide resistance of 7.5% (10). Fluoroquinolones have rapid bactericidal activity and suitable pharmacokinetic features. All of them can be given orally (in most cases both oral and parenteral), have a high volume of distribution and a wide extravascular penetration. They penetrate into inflammatory fluids 65–125% with respect to serum levels and concentrate in the cell reaching concentrations 7 to 14-fold higher than serum levels. Lung concentration on a whole, around 4-fold higher than serum levels. In recent years, newer fluoroquinolones have been developed that retain activity against Gram-negative organisms that is similar to the older fluoroquinolones but they have significantly improved activity against Gram-positive organisms particularly against pneumococci (7, 13, 14). The new fluoroquinolones gatifloxacin, gemifloxacin and moxifloxacin all demonstrate good *in vitro* activity, especially against gram positive organisms. Therefore these agents can be useful for treatment of bacterial respiratory infections and guidelines for the treatment of community acquired respiratory tract infections of several societies have been significantly modified. In those guidelines, fluoroquinolones appear as one of the preferred groups in outpatients, along with macrolides and tetracyclines, and are the drug-of-choice when penicillin resistant *S pneumoniae* are suspected (13, 15). Therefore, in this study, *in vitro* activities of ciprofloxacin, levofloxacin, gatifloxacin and moxifloxacin against common respiratory pathogens were examined.

Although the E-test is not approved by the NCCLS, studies show that E-test, agar dilution and broth microdilution methods are comparable in accuracy for susceptibility testing (16). In this study, *in vitro* susceptibilities of gatifloxacin, moxifloxacin, levofloxacin and ciprofloxacin were performed using E-test method. Since ciprofloxacin NCCLS published breakpoints are not available for categorical interpretation, ciprofloxacin MIC values for *S pneumoniae* strains were evaluated as described in previous studies (9). Also for *M catarrhalis* strains, no breakpoints were available in NCCLS so the same interpretation criteria of *H influenzae* were applied (10).

In the present study, all of *S pneumoniae* isolates collected were susceptible to the four floroquinolones. The MIC_{50} and MIC_{90} of ciprofloxacin were 1 mg/L and 2 mg/L, respectively (range 0.25-3 mg/L). In our region, there is no reported ciprofloxacin resistance in *S pneumoniae* even though this has been reported in many other countries across the world (10, 17). In the United States of America (USA) between 1994 to 1995, the prevalence of ciprofloxacin-resistant *S pneumoniae* isolates was already 1.2% (9). In Canada, from 1993 to 1997, the prevalence increased from 0 to 1.7% (18). In Spain, the percentage of ciprofloxacin-resistant *S pneumoniae* strains increased from 0.7% to 5% between 1991 and 1999, and it reached 23% among samples collected from Brooklyn, New York, USA between 1997 and 1999 (19, 20).

The new fluoroquinolones gatifloxacin and moxifloxacin, all demonstrate good *in vitro* activity against gram positive organisms particularly against pneumococci (7). In this study, MICs of moxifloxacin (MIC_{90} = 0.38 mg/L) and gatifloxacin (MIC_{90} =0.50 mg/L) against *S pneumoniae* were lower than the MICs of ciprofloxacin (MIC_{90} = 2 mg/L) and levofloxacin (MIC_{90} = 2 mg/L). As determined in this study, Liebowitz *et al* also found moxifloxacin was more active than levofloxacin against pneumococci (21). In this present study, for pneumococci, no resistance to new fluoroquinolones has been found; so far very little resistance has been reported in previous studies. In a European multicentre study which was done between 2000–2001, 99.6% of the *S pneumoniae* isolates collected were susceptible to moxifloxacin, gatifloxacin and levofloxacin. In that study, fluoroquinolone-non-susceptible isolates were collected from France (one isolate, moxifloxacin MIC 2 mg/L, gatifloxacin MIC 4 mg/L, levo-floxacin MIC 8 mg/L), Germany (one isolate, moxifloxacin MIC 2 mg/L, gatifloxacin MIC 4 mg/L, levofloxacin MIC 8 mg/L), Spain (one isolate, moxifloxacin MIC 4 mg/L, gati-floxacin MIC 8 mg/L, levofloxacin MIC 16 mg/L), and Italy (three isolates, moxifloxacin MICs 2–4 mg/L, gatifloxacin MICs 4–8 mg/L, levofloxacin MICs 8–16 mg/L) (22).

In the present study, all of *M catarrhalis* and *H influenzae* isolates were found susceptible to ciprofloxacin, levo-floxacin, gatifloxacin, and moxifloxacin. Ciprofloxacin de-monstrated the lowest MICs against these gram negative bacteria (for both; MIC_{90} = 0.032 mg/L). Newer agents, gatifloxacin and moxifloxacin offered no apparent advantages over ciprofloxacin and levofloxacin. Similar results demonstrating the excellent activity of fluoroquinolones *vs* community respiratory pathogens have been reported. In the SENTRY Antimicrobial Surveillance Programme, nearly all

(99.9%) *H influenzae* isolates were susceptible to ciprofloxacin (23). Also the Italian Epidemiological Survey group found no strains of *H influenzae* and *M catarrhalis* resistant to ciprofloxacin in Italy (24). In the European Multicenter Study done by Jones *et al* between 2000–2001, no *H influenzae* and *M catarrhalis* isolates were detected that were resistant to ciprofloxacin, levofloxacin, gatifloxacin and moxifloxacin (22).

Although, the four fluoroquinolones tested in this study showed good *in vitro* activity against the main respiratory pathogens, studies reporting resistance to these agents are likely to show an increase over time (25). Therefore continued monitoring of susceptibility patterns especially at local and national levels, will be required to detect any further changes in susceptibility.

REFERENCES

1. García-Garrote F, Cercenado E, Pedroviejo JM, Cuevas O, Bouza E. Comparative *in vitro* activity of the new quinolone gemifloxacin (SB-265805) with other fluoroquinolones against respiratory tract pathogens. J Antimicrob Chemother 2001; **47:** 681–4.
2. Baquero F, García-Rodríguez JA, García de Lomas J, Aguilar L. Antimicrobial resistance of 113 *Streptococcus pneumoniae* isolates from patients with respiratory tract infections in Spain: results of a 1–year (1996–1997) multicenter surveillance study. The Spanish Surveillance Group for Respiratory Pathogens. Antimicrob Agents Chemother 1999; **43:** 357–9.
3. Liñares J, de la Campa AG, Pallarés R. Fluoroquinolone resistance in *Streptococcus pneumoniae*. N Engl J Med 1999; **341:** 1546–7.
4. Felmingham D, Washington J. Trends in the antimicrobial susceptibility of bacterial respiratory tract pathogens findings of the Alexander Project 1992–1996. J Chemother 1999; **11** (**Suppl 1):** 5–21.
5. Chen DK, McGeer A, de Azavedo JC, Low DE. Decreased susceptibility of *Streptococcus pneumoniae* to fluoroquinolones in Canada. Canadian Bacterial Surveillance Network. N Engl J Med 1999; **341:** 233–9.
6. Critchley IA, Thornsberry C, Piazza G, Jones M, Hickey ML, Barth AL et al. Antimicrobial susceptibility of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* collected from five centers in Brazil, 1997-98. Clin Microbiol Infect 2000; **6:** 178–84.
7. Saravolatz LD, Leggett J. Gatifloxacin, gemifloxacin, and moxifloxacin: the role of 3 newer fluoroquinolones. Clin Infect Dis 2003; **37:** 1210–5.
8. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. 12th informational supplement M100-S12. Wayne, PA: NCCLS, 2002.
9. Brueggemann A, Coffman S, Rhomberg P, Huynh H, Almer L, Nilius A et al. Fluoroquinolone resistance in *Streptococcus pneumoniae* in United States since 1994–1995. Antimicrob Agents Chemother 2002; **46:** 680–8.
10. Zarakolu P, Soyletir G, Gur D, Unal S. Antimicrobial resistance patterns of respiratory pathogens: a local report from Turkey. Eur J Clin Microb Infect Dis 2003; **9:**1257–8.
11. Fine JM. Aetiology and incidence of community-acquired pneumonia. Infect Dis Clin Pract 1996; **5 (Suppl 4):** 127–35.
12. Garau J. Clinical perspectives on the management of community-acquired pneumonia. Diagn Microbiol Infect Dis 1996; **25:** 205–11.
13. García-Rodríguez JA, Bellido M. The role of fluoroquinolones in respiratory tract infections: community acquired pneumonia. Int J Antimicrob Agents 2000; **16:** 281–5.
14. Kowalski RP, Dhaliwal DK, Karenchak LM**,** Romanowsky EG, Mah FS, Ritterband DC et al. Gatifloxacin and moxifloxacin: an in vitro susceptibility comparison to levofloxacin, ciprofloxacin, and ofloxacin using bacterial keratitis isolates. Am J Ophthalmol 2003; **136:** 500–5.
15. Bartlett JG, Breiman RF, Mandell LA, File TM Jr. Community acquired pneumonia in adults: guidelines for management. The Infectious Diseases Society of America. Clin Infect Dis 1998; **26:** 811–38.
16. Perez-Vazquez M, Roman F, Varela MC, Canton R, Campos J. Activities of 13 quinolones by three susceptibility testing methods against a collection of *Haemophilus influenzae* isolates with different levels of susceptibility to ciprofloxacin: evidence for cross-resistance. J Antimicrob Chemother 2003; **51:** 147–51.
17. Kucukbasmaci O, Gonullu N, Aktas Z, Gurol D, Berkiten R. In vitro activity of telithromycin compared with macrolides and fluoroquinolones against *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis.* Int J Antimicrob Agents 2003; **22:** 497–501.
18. Chen DK, McGeer A, deAzavedo JC, Low DE. Decreased susceptibility of *Streptococcus pneumoniae* to fluoroquinolones in Canada.Canadian Bacteriarial Surveillance Network. N Engl J Med 1999; **341:** 233–9.
19. Drlica K, Zhao X. Fluoroquinolone-resistant *Streptococcus pneumoniae.* Rev Med Microbiol 2003; **14:** 95–103.
20. Quale J, Landman D, Ravishankar J, Flores C, Bratu S. *Streptococcus pneumoniae*, Brooklyn, New York: fluoroquinolone resistance at our doorstep. Emerg Infect Dis 2002; **8:** 594–7.
21. Liebowitz D, Slabbert M, Huisamen A. National surveillance programme on susceptibility patterns of respiratory pathogens in South Africa: moxifloxacin compared with eight other antimicrobial agents. J Clin Pathol 2003; **56:** 344–7.
22. Jones ME, Blosser-Middleton RS, Critchley IA, Karlowsky JA, Thornsberry C, Sahm DF. In vitro susceptibility of *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis*: a European multicenter study during 2000–2001. Eur J Clin Microb Infect Dis 2003; **9:** 590–9.
23. Gordon KA, Biedenbach DJ, Jones RN. Surveillance comparison of *Streptococcus pneumoniae* and *Haemophilus influenzae* susceptibilities from community-acquired respiratory tract infections and hospitalized patients with pneumonia: five-year results for the SENTRY Antimicrobial Surveillance Program. Diagn Microbiol Infect Dis 2003; **46:** 285–9.
24. Nicoletti G, Blandino G, Caccamo F, Friscia O, Schito AM, Speciale A. The Italian Epidemiological survey 1997–1999 antimicrobial susceptibility data of *Haemophilus influenzae, Haemophilus parainfluenzae* and *Moraxella catarrhalis* in Italy. Int J Antimicrob Agents 2002; **20:** 263–9.
25. Mlynarczyk G, Mlynarczyk A, Jeljaszewicz J. Epidemiological aspects of antimicrobic resistance in respiratory pathogens. Int J Antimicrob Agents 2001; **18:** 497–502.