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In vitro antibacterial activity of gemifloxacin and comparator compounds against common respiratory pathogens

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This study investigated the *in vitro* potency of the novel quinolone agent gemifloxacin (SB-265805), in comparison with other quionolones, β-lactams, macrolides and trimethoprim–sulphamethoxazole, against a panel of common respiratory pathogens. This panel comprised recent clinical isolates of *Streptococcus pneumoniae* (*n* = 347), *Haemophilus influenzae* (*n* = 256) and *Moraxella catarrhalis* (*n* = 184). Overall, the quinolones were highly active against *H. influenzae* and were the most potent agents against *M. catarrhalis*. Gemifloxacin was the most potent quinolone tested against all three species and was four- to 512-fold more potent against pneumococci than trovafloxacin, grepafloxacin, levofloxacin, ciprofloxacin, ofloxacin, gentamicin, cefuroxime, penicillin, ampicillin, clarithromycin, azithromycin or trimethoprim–sulphamethoxazole. Against 19 ofloxacin-intermediate and 52 ofloxacin-resistant strains of *S. pneumoniae*, gemifloxacin retained activity, and was the only agent tested with MICs of ≤0.5 mg/L. The results of this study demonstrate the excellent *in vitro* antibacterial activity of gemifloxacin against pathogens commonly associated with respiratory tract infections and suggest that gemifloxacin has significant potential in the treatment of such infections, including those caused by pneumococci considered resistant to other quinolones.

Introduction

Community-acquired respiratory tract infections account for more antimicrobial prescriptions than any other non-hospital infection. In 1998, over 247 million courses of antimicrobials were prescribed in the USA for outpatient treatment of such infections.[1] The pathogens most commonly associated with bacterial infections of the upper and lower respiratory tract, including otitis media, acute sinusitis, acute exacerbations of chronic bronchitis and community-acquired pneumonia are *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*.[2,3] It is common practice to treat respiratory infections empirically but, owing to the increase in antimicrobial resistance associated with these organisms, the range of appropriate antimicrobial therapy may be compromised. For example, in 1994–1995 it was reported that 23.6% of *S. pneumoniae* clinical isolates were not susceptible to penicillin: 14.1% were of intermediate susceptibility and 9.5% displayed high-level resistance.[4] The results of a 1996–1997 surveillance study in the USA reported that 33.6% of *S. pneumoniae* were either penicillin-intermediate or -resistant.[5] The significance of penicillin-resistant *S. pneumoniae* is amplified by the increasing incidence of cross-resistance to other antimicrobials, including cephalosporins, macrolides, tetracycline, chloramphenicol and trimethoprim–sulphamethoxazole.[4]Although much of the focus has been on pneumococcal resistance, the number of β-lactamase-producing *M. catarrhalis* and *H. influenzae* has also increased over the past two decades. In 1974, several infections caused by β-lactamase-producing *H. influenzae* were reported.[6,7] Ten years after these initial reports, the prevalence of β-lactamase-producing *H. influenzae* had increased to 15.2%;[8] in 1993, the prevalence had increased to 33%[9] and, most recently, it was reported at 33.5%.[10] The problem is significantly greater among *M. catarrhalis*, where >90% produce β-lactamase.[10–12] Alternative antibacterial agents, such as the macrolides, are of limited empirical use owing to their marginal activity against *H. influenzae* and decreased activity against multiresistant

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penicillin-intermediate and -resistant pneumococci.[13] With the increase in resistance and the limited number of antimicrobials offering an appropriate spectrum of activity for empirical use, the need for new therapeutic agents is pressing.

Gemifloxacin is a novel quinolone antibacterial agent that is highly potent against the respiratory tract pathogens *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*.[14] To confirm these findings among clinical isolates, and to compare the potency of gemifloxacin with that of other agents currently used in the treatment of community-acquired respiratory tract infection, the *in vitro* activity of these antimicrobials was determined against recent clinical isolates of *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* and against a collection of quinolone-intermediate (ofloxacin MIC 4 mg/L) and quinolone-resistant (ofloxacin MIC $\geqslant$ 8 mg/L) isolates of *S. pneumoniae*.

Materials and methods

Isolates

Recent clinical isolates of *S. pneumoniae* and *H. influenzae* were obtained from the 1997 ALERT programme (Laboratory Specialists, Inc., Westlake, OH, USA)—a multicentre surveillance study involving 47 USA hospitals. Isolates of *M. catarrhalis* were obtained from five sources: the ALERT surveillance study; The Bryn Mawr Hospital, Bryn Mawr, PA, USA; Evanston Hospital, Evanston, IL, USA; UCSF Stanford Health Care, Stanford, CA, USA; and SmithKline Beecham Clinical Laboratories, West Norriton, PA, USA. The ofloxacin-resistant isolates were collected from the following sources: The Alexander Project[3,12] (47 isolates; 1992–1998), SENTRY[15] (seven isolates; 1996), SmithKline Beecham Clinical Laboratories (one isolate; 1997), SPAR[16] (nine isolates; 1996), University of Iowa (three isolates; 1996) and ALERT[17] (four isolates; 1997).

Broth microdilution

Microtitre susceptibility plates, prepared by Sensititre, were used for MIC testing (AccuMed International Ltd, Westlake, OH, USA; lot numbers CMP5ASMK-8331 and -8423, and CMP4BSMK-8351 and -8451). These plates contained the following compounds in serial doubling dilutions: gemifloxacin (0.001–256 mg/L), trovafloxacin (0.016–16 mg/L), grepafloxacin (0.016–16 mg/L), levofloxacin (0.016–16 mg/L), ciprofloxacin (0.016–16 mg/L), ofloxacin (0.016–64 mg/L), cefuroxime (0.06–64 mg/L), penicillin (0.016–64 mg/L), ampicillin (0.06–64 mg/L), clarithromycin (0.016–16 mg/L), azithromycin (0.06–64 mg/L) and trimethoprim–sulphamethoxazole (0.06/1.14–64/1216 mg/L). In accordance with the recommended procedure of the manufacturer, each plate was inoculated with 100 μL/well of a single test isolate which resulted in a final inoculum density of 5×10^5 cfu/mL.[18] The *M. catarrhalis* isolates were tested in cation-adjusted Mueller–Hinton broth (BBL, Cockeysville, MD, USA). For the *S. pneumoniae* isolates, this broth was supplemented with 5% lysed horse blood (BBL). *H. influenzae* isolates were tested using Haemophilus Test Medium (HTM; Dade Behring, West Sacramento, CA, USA).[19] Colony counts were performed at random to ensure the appropriate inoculum density was obtained. The Microlab AT Plus 2 (Hamilton Co., Reno, NV, USA) was used to add the inoculum to the microtitre plate. Following inoculation, the plates were covered and incubated at 35°C in ambient air for 20–24 h. A 10 μL aliquot of inoculum was plated on trypticase soy agar containing 5% sheep blood to determine the purity of the final test inoculum. Chocolate agar (BBL) was used to determine the purity of *H. influenzae* cultures. The production of β-lactamase was determined for the *H. influenzae* isolates using the cefinase disc test obtained from BBL.

The MIC was determined as the lowest concentration of drug that inhibited visible growth of the test isolates. For trimethoprim–sulphamethoxazole, the MIC was defined as the concentration that produced an 80–90% decrease in growth compared with the positive growth control. Individual MIC data were summarized and reported as MIC range, MIC_{50} and MIC_{90}.

Quality control

The following quality control organisms were included on each day of testing: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247, *H. influenzae* ATCC 49766 and *E. coli* ATCC 35218. Results for that day were only accepted if the quality control values were within the acceptable limits as established by the NCCLS.[20] Quality control limits tentatively approved by the NCCLS in January 1999, were used for gemifloxacin.

Results

The MIC distributions of gemifloxacin and the comparator agents are shown in Table I. Because of out-of-range quality control results, only 299 penicillin and 278 clarithromycin MIC results for *S. pneumoniae* and 208 azithromycin MIC results for *H. influenzae* were included in the evaluation. Inadequate growth of individual test isolates of *M. catarrhalis* also meant that only 159 grepafloxacin and 181 azithromycin MIC results were included for this organism. Judging from the results of the cefinase disc test, 88 of the 256 *H. influenzae* isolates were β-lactamase producers.

Of the 347 *S. pneumoniae* isolates tested, 65 were penicillin-intermediate (MIC 0.125–1 mg/L), 96 were penicillin-resistant (MIC $\geqslant$ 2 mg/L) and nine were ofloxacin-

Table I. Antimicrobial activity (mg/L) of gemifloxacin and comparator compounds against a panel of recent clinical isolates of *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*

	S. pneumoniae (n = 347)			*H. influenzae* (n = 256)			*M. catarrhalis* (n = 184)		
Antimicrobial	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}	MIC range
Gemifloxacin	0.016	0.03	0.004–0.06	0.004	0.008	0.002–0.016	0.008	0.008	0.004–0.016
Trovafloxacin	0.12	0.12	≤0.016–0.5	≤0.016	≤0.016	≤0.016–0.06	≤0.016	≤0.016	≤0.016–0.03
Grepafloxacin[a]	0.12	0.25	≤0.016–0.5	≤0.016	≤0.016	≤0.016–0.06	≤0.016	≤0.016	≤0.016–0.03
Levofloxacin	1	1	0.25–2	≤0.016	≤0.016	≤0.016–0.06	0.03	0.06	0.03–0.06
Ciprofloxacin	1	2	0.12–4	≤0.016	≤0.016	≤0.016–0.03	0.03	0.03	≤0.016–0.03
Ofloxacin	2	2	0.25–8	≤0.06	≤0.06	≤0.06–0.12	≤0.06	0.12	≤0.06–0.25
Cefuroxime	0.25	8	≤0.06–64	1	2	0.25–8	1	2	0.12–4
Penicillin[b]	0.06	2	≤0.016–4	0.5	>16	0.06–>16	8	16	≤0.016–>16
Ampicillin	0.06	4	≤0.06–16	0.25	>64	0.12–>64	2	4	≤0.06–16
Clarithromycin[b]	0.03	8	≤0.06–>16	8	16	0.12–>16	0.06	0.12	≤0.016–0.25
Azithromycin[a,c]	0.06	16	≤0.06–>64	1	2	0.25–8	≤0.06	≤0.06	≤0.06
Trimethoprim–sulphamethoxazole	0.5	8	≤0.06–32	0.25	8	≤0.06–32	0.25	0.5	0.12–8

[a]Grepafloxacin and azithromycin results are based on 159 and 181 of the *M. catarrhalis* isolates, respectively.
[b]Penicillin and clarithromycin were tested against 299 and 278 of the *S. pneumoniae* isolates, respectively.
[c]Azithromycin results are based on 208 of the *H. influenzae* isolates.

Table II. *In vitro* activity of gemifloxacin and comparator compounds against ofloxacin-susceptible, -intermediate and -resistant strains of *S. pneumoniae*

Ofloxacin susceptibility	n	MIC (mg/L)				
		gemifloxacin	trovafloxacin	ciprofloxacin	ofloxacin	penicillin
Susceptible	338					
MIC range		0.004–0.03	0.03–0.25	0.12–2	0.5–2	0.03–4
MIC_{50}		0.03	0.12	1	2	0.06
MIC_{90}		0.03	0.12	2	2	4
Intermediate	19					
MIC range		0.03–0.12	0.06–0.5	2–8	4	0.008–2
MIC_{50}		0.03	0.25	4	4	0.016
MIC_{90}		0.06	0.5	8	4	1
Resistant	52					
MIC range		0.03–0.5	0.5–16	4–>64	8–>64	≤0.016–4
MIC_{50}		0.25	4	32	32	1
MIC_{90}		0.25	8	64	32	2

intermediate or -resistant (ofloxacin MIC ≥ 4 mg/L). Data comparing the 338 ofloxacin-susceptible isolates with 19 ofloxacin-intermediate and 52 ofloxacin-resistant isolates are shown in Table II.

Discussion

In this study of a large number of bacterial respiratory pathogens, the potency of gemifloxacin was found to be equal or superior to that of the comparator agents. Gemifloxacin (MIC_{90} 0.03 mg/L) was four- to 256-fold more potent than the other quinolones (MIC_{90} 0.12–>64 mg/L) and 64- to 512-fold more potent than the non-quinolone comparators (MIC_{90} 2–16 mg/L) for quinolone-susceptible and -intermediate *S. pneumoniae*. Although the quinolones were generally highly potent against *H. influenzae*, gemifloxacin was the most potent compound tested against this pathogen (MIC_{90} 0.008 mg/L). These findings are consistent with the results of other studies, which found

that gemifloxacin had excellent *in vitro* activity against *H. influenzae*.[14,21]

The range of MICs obtained for gemifloxacin against *M. catarrhalis* was 0.004–0.016 mg/L. Based on MIC_{90}s, gemifloxacin (0.008 mg/L), trovafloxacin, grepafloxacin (≤0.016 mg/L) and azithromycin (≤0.016 mg/L) were among the most potent compounds tested against this organism. The MIC_{90}s of the other quinolones tested were: ciprofloxacin, 0.03 mg/L; levofloxacin, 0.06 mg/L; and ofloxacin, 0.125 mg/L. The quinolones were generally more potent than the other classes of compound tested.

Numerous studies have reported on the incidence of quinolone-resistant *S. pneumoniae*.[3,5,12] Although the frequency is considered relatively low, an increase in the prevalence of these resistant isolates could potentially restrict the use of quinolones in the treatment of infections caused by this organism. As shown in this study, gemifloxacin maintains its potency against *S. pneumoniae* strains considered resistant to other quinolones (Table II). The highest MIC of gemifloxacin was 0.5 mg/L for these strains, as compared with 16 mg/L of trovafloxacin and >64 mg/L of ofloxacin and ciprofloxacin. The mechanisms of resistance in these quinolone-resistant strains and the reasons for the maintained potency of gemifloxacin against *S. pneumoniae* are discussed by Broskey *et al.*[22] and Morrissey & George[23] in papers within this Supplement.

The results of this study demonstrated that gemifloxacin has excellent *in vitro* potency against recent clinical isolates of *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*. Of particular note was its good potency against pneumococci, including strains resistant to all other quinolones tested. These findings suggest that gemifloxacin has significant potential for use in the treatment of respiratory tract infections caused by these common pathogens.

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