Contemporary Activity of Gemifloxacin and Other Fluoroquinolones Tested against *Streptococcus pneumoniae* Isolated During 1999 - 2004 in Europe and the Americas

DJ BIEDENBACH, JE ROSS, HS SADER, TR FRITSCHE, RN JONES
JMI Laboratories, North Liberty, IA, USA

ECCMID 2005
JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, fax 319.665.3371
ronald-jones@jmilabs.com

AMENDED ABSTRACT

Objectives: To determine the potency of gemifloxacin (GEMI) tested against *S. pneumoniae* (SPN) for the years 1999-2004. The evaluation of GEMI compared to other currently marketed fluoroquinolones (FQ) will also be made including mechanisms of resistance (R).

Methods: During a six year period (1999-2004), a total of 9,369 SPN isolates were collected from medical centers on three continents and tested for antimicrobial susceptibility (S) using reference CLSI/NCCLS broth microdilution methods and interpretive criteria (M100-S15, 2005). The antimicrobial agents tested included five FQs: GEMI, ciprofloxacin (CIPRO), levofloxacin (LEVO), gatifloxacin (GATI) and moxifloxacin (MOXI). Analysis of the quinolone resistance determining region (QRDR) was performed for 35 FQ- R strains (LEVO MIC at >4 mg/L).

Results: The activity of GEMI against SPN over a six year period is shown in the table:

Organism (no. tested)	GEMI MIC (mg/L)		% by category		
Year (no. tested)	50%	90%	S	I	R
1999 (812)	<=0.03	<=0.03	99.5	0.4	0.1
2000 (825)	<=0.03	<=0.03	99.5	0.2	0.2
2001 (2,891)	0.016	0.03	99.4	0.4	0.2
2002 (2,948)	0.016	0.03	99.3	0.6	0.1
2003 (890)	0.016	0.03	100.0	0.0	0.0
2004 (1,003)	0.016	0.03	99.2	0.7	0.1
All years (9,369)	0.016	0.03	99.5	0.3	0.2

During the six years, the rank order of potency (MIC_{90}, mg/L) for the FQs was GEMI (0.03) > MOXI (0.12-0.25) > GATI (0.5) > LEVO (1-2) > CIPRO (2). All FQ median and modal potencies remained stable over the monitored interval. SPN isolates with CIPRO MIC values >=4 mg/L ranged from 1.6-2.1% with no detectable trend towards increasing R across all regions. The most common QRDR mutations among strains with CIPRO and LEVO MIC values >=4 mg/L were: *gyrA* (S81F), *parC* (S79F or T, D83N and K137N) and *parE* (I460V). Against these isolates, GEMI maintained the most potent activity at $MIC_{50/90}$ values of 0.25/1 mg/L and a MIC range of only ≤ 0.12 - 2 mg/L.

Conclusions: Among the FQs tested, GEMI was clearly the most potent agent and remains a valuable candidate for treating multi-drug R SPN, an increasingly observed respiratory-tract pathogen. During the study years, FQ-R did not increase significantly and S rates to GEMI remained at >99.2% and was unrelated to increasing R among beta-lactam and macrolide antimicrobials.

INTRODUCTION

Many newer fluoroquinolones have been developed that have excellent clinical efficacy and greater potencies compared to older generation compounds against respiratory tract pathogens. Among the common pathogens that cause community-acquired pneumonia which include *S. pneumoniae*, *H. influenzae*, *M. catarrhalis* and atypical pathogens, the pneumococcus is capable of harbouring a multitude of resistance mechanisms which affect a broad range of antimicrobial classes. Penicillin resistance has been reported at variable rates from region to region or between countries and can be over 50%. Among a large global collection of pneumococci from the SENTRY Antimicrobial Surveillance Program (1997 - 2003), 17.6% of strains were highly resistant to penicillin (MIC, ≥ 2 mg/L) and erythromycin. Nearly 6% of the isolates were resistant to five drug classes (penicillin, macrolides, lincosamides, tetracyclines and trimethoprim/sulfamethoxazole). A study in Europe found 22.2% of the *S. pneumoniae* tested were resistant to ≥ three drug classes. With the increasing rates of resistance among pneumococci, the fluoroquinolone class has become a popular alternative therapy.

In the United States, three newer fluoroquinolones have received the indication for treating respiratory tract infections in adult patients (moxifloxacin, gatifloxacin and gemifloxacin). Gemifloxacin is highly active against *S. pneumoniae* including multidrug-resistant strains and has been approved for the treatment of acute bacterial exacerbations of chronic bronchitis and community-acquired pneumonia. Clinical trials have shown superior outcomes compared to other drug classes and some older fluoroquinolones. This study will evaluate the potencies of fluoroquinolones including gemifloxacin against recent strains of *S. pneumoniae* collected from 1999 - 2004 on three continents. A subset analysis of strains with fluoroquinolone-resistance will have quinolone resistance determining region (QRDR) sequenced for mutations.

MATERIALS AND METHODS

The gemifloxacin surveillance network collected a total of 9,369 viable isolates of *S. pneumoniae* during 1999 - 2004 from Europe, North America and Latin America. Isolates were tested against numerous antimicrobial classes including fluoroquinolones. Each year, pneumococci were tested against gemifloxacin, gatifloxacin and ciprofloxacin. Levofloxacin was tested during 2000 - 2004 and moxifloxacin was tested during 2001 - 2004. The isolates were tested using reference MIC methods according to CLSI/NCCLS recommendations in broth microdilution panels supplied by TREK Diagnostics (Cleveland, OH, USA). Testing was performed using a standard 0.5 McFarland inoculum concentration with 3 - 5% lysed horse blood supplemented Mueller-Hinton broth as a growth medium.

A subset of 35 isolates with ciprofloxacin MIC results ≥ 4 mg/L were selected from 24 different medical centers located in all three regions for determination of QRDR mutations. Amplified PCR products of genes *parC, parE, gyrA* and *gyrB* were sequenced in both strands by the dideoxy-chain termination method with a Perkin Elmer Biosystems 377 DNA sequencer and sequence analysis performed using the Lasergene software package (DNAstar, Madison, WI ,USA).

RESULTS

Table 1. Frequency distribution for five fluoroquinolones tested against *S. pneumoniae* (9,369 isolates) over six years (1999 - 2004) in Europe, North America and Latin America.

		MIC (mg/L)								
Year (no. tested)	Antimicrobial agent	≤0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	>4
1999 (812)	Gemifloxacin	NT[a]	98.3	99.1	99.5[b]	99.9	100.0	-	-	-	-
	Moxifloxacin	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
	Gatifloxacin	NT	0.4	0.7	7.9	86.8	99.4	99.5	99.5	99.9	100.0
	Levofloxacin	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
	Ciprofloxacin	NT	NT	NT	NT	1.8	12.7	80.0	98.3	-	-
2000 (825)	Gemifloxacin	NT	98.2	99.4	99.5	99.8	100.0	-	-	-	-
	Moxifloxacin	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
	Gatifloxacin	NT	0.4	0.7	9.3	86.3	99.3	99.6	99.8	99.9	100.0
	Levofloxacin	NT	0.1	0.2	0.5	0.7	37.9	98.3	99.6	99.8	100.0
	Ciprofloxacin	NT	NT	NT	NT	1.2	14.4	76.5	97.5	-	-
2001 (2,891)	Gemifloxacin	NT	NT	NT	99.4	99.8	99.9	100.0	-	-	-
	Moxifloxacin	NT	0.6	13.7	89.0	98.6	99.1	99.3	99.8	99.9	100.0
	Gatifloxacin	NT	0.1	0.4	7.2	85.4	98.8	99.3	99.4	99.8	100.0
	Levofloxacin	NT	-	0.1	0.2	0.7	23.9	97.3	99.3	99.3	100.0
	Ciprofloxacin	-	-	0.1	0.2	0.7	8.4	68.1	95.5	-	-
2002 (2,948)	Gemifloxacin	NT	NT	NT	99.3	99.8	99.9	100.0	-	-	-
	Moxifloxacin	NT	1.7	19.4	88.0	98.5	98.7	99.0	99.7	99.9	100.0
	Gatifloxacin	NT	0.4	1.0	8.9	84.6	98.6	99.0	99.2	99.9	100.0
	Levofloxacin	NT	0.2	0.2	0.3	1.2	35.7	98.0	99.0	99.1	100.0
	Ciprofloxacin	NT	0.2	0.3	0.3	0.8	11.0	71.1	96.6	98.9	100.0
2003 (890)	Gemifloxacin	73.1	98.2	99.9	100.0	-	-	-	-	-	-
	Moxifloxacin	NT	1.2	11.2	79.0	99.6	99.8	99.9	100.0	-	-
	Gatifloxacin	NT	0.2	0.8	6.6	70.9	99.1	99.9	100.0	-	-
	Levofloxacin	-	-	0.2	0.3	0.9	17.0	88.3	99.9	99.9	100.0
	Ciprofloxacin	-	-	0.2	0.3	0.6	7.8	49.9	89.1	98.4	100.0
2004 (1,003)	Gemifloxacin	80.8	97.4	98.9	99.2	99.9	100.0	-	-	-	-
	Moxifloxacin	NT	0.9	21.3	93.6	98.4	98.8	98.9	99.9	100.0	-
	Gatifloxacin	NT	0.2	0.6	10.3	84.6	98.6	98.8	98.9	99.9	100.0
	Levofloxacin	NT	0.1	0.1	0.2	0.5	28.9	98.3	98.8	98.8	100.0
	Ciprofloxacin	NT	0.1	0.1	0.1	0.6	13.6	76.1	98.0	98.7	100.0

a. NT = antimicrobial was not tested during this study year or at this concentration.
b. Percentages in blue represent the susceptibility rates for antimicrobials with CLSI/NCCLS breakpoint criteria (M100-S15) for *S. pneumoniae*.

- All fluoroquinolones with CLSI/NCCLS breakpoint criteria had excellent in vitro activity against *S. pneumoniae* isolates from Europe, North America and Latin America with susceptibility percentages ≥ 99% during all six years (Table 1).
- As a marker for decreased fluoroquinolone-susceptibility, *S. pneumoniae* isolates with ciprofloxacin MIC values ≥ 4 mg/L increased during the years 1999 (1.7%), 2000 (2.5%) and 2001 (4.5%) then declined slightly in 2002 (3.4%) and 2004 (2.0%). During 2003, the highly elevated rate of 10.9% was mainly due to a clone in an Italian hospital that accounted for 24.7% of the isolates with ciprofloxacin MIC values of ≥ 4 mg/L. Overall, rates were highest in Europe (5.2%) compared to Latin America (4.1%) and North America (3.4%).
- The potencies of the fluoroquinolones against isolates collected in 2003 and 2004 are shown in Table 2, separated by penicillin-susceptibility categories. Gemifloxacin was the most potent fluoroquinolone tested (MIC_{90}, 0.03 mg/L) > moxifloxacin (0.12 - 0.25 mg/L) > gatifloxacin (0.25 - 0.5 mg/L) > levofloxacin (1 - 2 mg/L) > ciprofloxacin (2 - 4 mg/L). Penicillin susceptibility had no effect on fluoroquinolone MIC values.
- *S. pneumoniae* isolates with decreased susceptibility to ciprofloxacin (MIC, ≥ 4 mg/L) were less susceptible to penicillin (64.6%), erythromycin (60.8%) and clindamycin (80.7%) compared to wild-type strains with susceptibility rates of 70.3%, 77.1% and 89.0%, respectively. No correlation was shown for tetracycline or trimethoprim/sulfamethoxazole (data not shown).
- Gemifloxacin (MIC_{90}, ≤ 0.25 mg/L) was the most potent agent tested against *S. pneumoniae* isolates with elevated ciprofloxacin MIC values (≥ 4 mg/L) compared to moxifloxacin (2 mg/L), gatifloxacin (4 mg/L) and levofloxacin (> 4 mg/L).
- Table 3 shows that against 35 isolates with levofloxacin MIC values of > 4 mg/L, gemifloxacin had the lowest MIC results (MIC_{90}, 0.25 mg/L) compared to moxifloxacin (2 mg/L) and gatifloxacin (4 mg/L). The most common mutations were Ser81-Phe (*gyrA*), Ser79-Phe or Tyr (*parC*) and Ile460-Val (*parE*). Typically, strains with multiple mutations (four) had the highest fluoroquinolone MIC values.

Table 2. Distribution of fluoroquinolone MIC values based upon susceptibility to penicillin against *S. pneumoniae* isolates in Europe, North America and Latin America (2003 - 2004).

		2003			2004		
Penicillin category	Antimicrobial agent	MIC_{50}	MIC_{90}	% susceptible[a]	MIC_{50}	MIC_{90}	% susceptible[a]
Susceptible	Gemifloxacin	≤0.016	0.03	100.0	≤0.016	0.03	98.8
	Moxifloxacin	0.12	0.25	99.8	0.12	0.12	98.4
	Gatifloxacin	0.25	0.5	99.8	0.25	0.5	98.3
	Levofloxacin	1	2	99.8	1	1	98.3
	Ciprofloxacin	2	4	-[b]	1	2	-
Intermediate	Gemifloxacin	≤0.016	0.03	100.0	≤0.016	0.03	100.0
	Moxifloxacin	0.12	0.25	100.0	0.12	0.12	99.4
	Gatifloxacin	0.25	0.5	100.0	0.25	0.25	99.4
	Levofloxacin	1	2	100.0	1	1	99.4
	Ciprofloxacin	1	4	-	1	2	-
Resistant	Gemifloxacin	≤0.016	0.03	100.0	≤0.016	0.03	100.0
	Moxifloxacin	0.12	0.25	100.0	0.12	0.12	100.0
	Gatifloxacin	0.25	0.5	100.0	0.25	0.5	100.0
	Levofloxacin	1	1	100.0	1	1	100.0
	Ciprofloxacin	2	4	-	1	2	-

a. Susceptibility percentages were based upon CLSI/NCCLS breakpoint criteria (M100-S15).
b. No susceptibility breakpoints have been established.

Table 3. The activity of three fluoroquinolones tested against 35 *S. pneumoniae* isolates with resistant levofloxacin MIC values (> 4 mg/L) and mutations in the QRDR.

		QRDR mutations				MIC (mg/L)		
Strain #	Origin	*gyrA*	*gyrB*	*parC*	*parE*	Gemifloxacin	Moxifloxacin	Gatifloxacin
1	USA	Ser81-Phe	-	Lys137-Asn	Ile460-Val	≤0.12	1	2
2	USA	Ser81-Phe	-	-	Ile460-Val	≤0.12	2	4
3	Belgium	Ser81-Phe	-	Ser79-Phe	Ile460-Val	≤0.12	2	4
4	France	Ser81-Phe	-	Ser79-Phe	-	≤0.12	1	2
5	Italy	Ser81-Phe	-	Ser79-Phe	Ile460-Val	≤0.12	2	4
6	USA	Ser81-Phe	-	Ser79-Tyr + Lys137-Asn	-	≤0.12	2	4
7	USA	Ser81-Phe	-	Ser79-Tyr	-	≤0.12	2	4
8	USA	Ser81-Phe	-	Ser79-Phe	-	≤0.12	2	4
9	USA	-	-	Ser79-Phe + Lys137-Asn	-	≤0.12	2	4
10	Italy	Ser81-Phe	-	Ser79-Phe	Ile460-Val	≤0.12	2	4
11	France	Ser81-Phe	-	Ser79-Phe	Ile-460-Val	0.25	2	4
12	Canada	-	-	Ser79-Phe	-	0.25	2	4
13	France	-	-	-	Ile460-Val	0.25	2	4
14	USA	Ser81-Phe	-	Ser79-Phe	-	0.25	2	4
15	Canada	Ser81-Phe	-	Ser79-Phe	-	0.25	2	4
16	Italy	Ser81-Phe	-	Ser79-Phe + Lys137-Asn	Ile460-Val	0.25	2	4
17	Italy	Ser81-Phe	-	Ser79-Tyr	Ile460-Val	0.25	4	>4
18	USA	Ser81-Phe	-	Ser79-Phe	Ile460-Val	0.25	2	4
19	USA	-	-	Ser79-Tyr	Ile460-Val	0.25	2	4
20	USA	Ser81-Phe	-	Ser79-Tyr	-	0.25	2	4
21	USA	Ser81-Phe	-	-	Ile460-Val	0.25	2	4
22	Italy	Ser81-Phe	-	Asp83-Gly	Ile460-Val	0.25	2	4
23	USA	Ser81-Phe	-	Asp83-Asn	-	0.25	2	4
24	USA	Ser81-Phe	-	Asn91-Asp	-	0.25	4	4
25	Italy	Ser81-Phe	-	-	-	0.25	2	4
26	Italy	Ser81-Phe	-	Ser79-Phe	Ile460-Val	0.25	4	>4
27	Chile	-	Asp435-Ile	Ser79-Phe	-	0.5	2	>4
28	Chile	-	Asp435-Ile	Ser79-Phe	-	0.5	2	>4
29	Canada	Ser81-Phe	-	Ser79-Phe	-	0.5	4	>4
30	Canada	Glu85-Lys	-	Ser79-Phe	Ile460-Val	0.5	4	>4
31	USA	Ser81-Phe + Ala17-Thr	-	Ser79-Phe	-	0.5	2	4
32	Canada	Glu85-Lys	-	Ser79-Phe + Lys137-Asn	Ile460-Val	1	>4	>4
33	Switzerland	Ser81-Phe	-	Ser79-Phe	-	1	>4	>4
34	USA	-	Val432-Asp	Ser79-Phe + Gly77-Glu	Ile460-Val	1	4	>4
35	USA	Ser81-Phe + Glu85-Lys	-	Ser79-Phe + Asp83-Tyr	-	>1	>4	>4

CONCLUSIONS

- Gemifloxacin was the most potent fluoroquinolone tested against *S. pneumoniae* isolates from three continents during 2001 - 2004.
- Penicillin and macrolide resistance did not adversely affect the potencies of fluoroquinolones. However, strains of pneumococci with elevated fluoroquinolone MIC values were more likely to be resistant to other drug classes.
- A relatively greater gemifloxacin potency (eight-fold) was maintained against isolates with QRDR mutations compared to moxifloxacin and gatifloxacin.

SELECTED REFERENCES

Appelbaum PC, Gillespie SH, Burley CJ, Tillotson GS. (2004). Antimicrobial selection for community-acquired lower respiratory tract infections in the 21st century: A review of gemifloxacin. *International Journal of Antimicrobial Agents* 23:533-546.

Chen DK, McGeer A, de Azavedo JC, Low DE. (1999). Decreased susceptibility of *Streptococcus pneumoniae* to fluoroquinolones in Canada. Canadian Bacterial Surveillance Network. *New England Journal of Medicine* 341:233-239.

Clinical and Laboratory Standards Institute. (2005). *Performance standards for antimicrobial susceptibility testing, 15th informational supplement M100-S15*. Wayne, PA:CLSI.

Felmingham D, Shackcloth J, Tillotson G. (2004). BSAC Respiratory Resistance Surveillance Programme (2002 -2003): Comparative susceptibility of *Streptococcus pneumoniae*, cultured from patients in Great Britain and Ireland with community-acquired lower respiratory tract infection, to gemifloxacin. *Journal of Antimicrobial Chemotherapy* 54:698-699.

File TM Jr, Tillotson GS. (2004). Gemifloxacin: A new, potent fluoroquinolone for the therapy of lower respiratory tract infections. *Expert Reviews Antimicrobial Infection Therapy* 2:831-843.

Jones RN. (2002). Microbiology of newer fluoroquinolones: Focus on respiratory pathogens. *Diagnostic Microbiology and Infectious Disease* 44:213-220.