A Recent Evaluation of *In Vitro* Activity of Gemifloxacin (GEM), Moxifloxacin (MOX), Gatifloxacin (GAT) and Levofloxacin (LEV) Against 2517 *Streptococcus pneumoniae* (*SP*) Isolates Recovered From Sterile Body Sites: The FACTS Study, 2000–2001

D.E. Low,[1] D.J. Hoban,[2] S.K. Bouchillon,[2] J.L. Johnson,[2] S.B. Min,[3] D.L. Butler,[3] K.A. Saunders[3] & J.A. Poupard[3]

[1]Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada. [2]Laboratories International for Microbiology Studies, Rolling Meadows, IL, USA. [3]Microbiology, GlaxoSmithKline, Collegeville, PA, USA.

Revised Abstract

Background: The new fluoroquinolones continue to demonstrate excellent *in vitro* activity against *SP* isolates from respiratory infections with previously reported MIC_{90}s of 0.03, 0.25, 0.5 and 1.0 µg/ml for GEM, MOX, GAT and LEV, respectively. *SP* is also an important pathogen in invasive infections such as bacteremia and meningitis but fluoroquinolone activity in these strains of *SP* is not well documented. This study reports on the activity of new fluoroquinolones against *SP* isolates from blood, cerebrospinal fluid (CSF), paracentesis fluid and other sterile body sites (SBSs). **Method:** Susceptibility determinations were performed on 2517 *SP* isolates from SBSs in a two-phase study. The first phase measured penicillin (PEN), erythromycin (ERY), clindamycin (CC), GEM and LEV activity by disk diffusion and/or by Etest methodology. Isolates with a MIC of ≥1.5 µg/ml to LEV (indicating possible mutation[s]) were tested in phase II against GEM, MOX, GAT and LEV using the Etest methodology. Interpretations were made based on NCCLS guidelines (analysis for GEM is based on susceptible, intermediate and resistant breakpoints of ≤0.25, 0.5 and ≥1.0 µg/ml, respectively). **Results:** Phase I results showed *SP* resistance rates of 15.8%, 21.5% and 4.6% for SBS isolates against PEN, ERY and CC, respectively. GEM and LEV *SP* MIC_{90}s (µg/ml) and resistance rates in phase I were 0.047/0.1% and 1.0/0.4%, respectively. *SP* isolates with a LEV MIC of ≥1.5 µg/ml (137 isolates) tested in phase II exhibited MIC_{90}s (µg/ml) and resistance levels of 0.125/1.5%, 0.75/7.3%, 0.75/6.6% and 3.0/7.3% to GEM, MOX, GAT and LEV, respectively. The percentage of phase II *SP* isolates that exhibited MICs of ≥32 µg/ml for GEM, MOX, GAT and LEV were 0.0%, 1.5%, 2.2% and 6.6%, respectively. **Conclusion:** PEN and ERY resistance rates were similar to those observed in previous studies. *SP* isolates from SBSs demonstrated the lowest resistance and MIC_{90}s to GEM in both phase I and II testing. *SP* isolates with MICs of ≥32 µg/ml were found most often for LEV.

Introduction

It is estimated that *Streptococcus pneumoniae* accounts for 3000 cases of meningitis, 50,000 cases of bacteremia, 500,000 cases of pneumonia and more than 7,000,000 cases of otitis media annually and is the leading cause of illness and death in young children and the elderly worldwide.[1] Because of the importance of this resourceful pathogen and the alarming increase in resistance to penicillin and macrolides, the medical community is turning to other therapeutic agents to treat this pathogen. Fluoroquinolones are now recommended as first-line therapy against *S. pneumoniae* in many infections, especially respiratory stains of *S. pneumoniae* that are resistant to penicillin and macrolides. Fluoroquinolone activity is well documented in respiratory tract strains of *S. pneumoniae* but not nearly as well documented in sterile body sites such as blood, cerebrospinal fluid and others.

Objectives

Approximately 7319 isolates of *S. pneumoniae* from the USA and Canada were evaluated in the FACTS study. Primary screening was performed to determine the current susceptibility and resistance of *S. pneumoniae* to penicillin, macrolides and quinolones. Further testing was performed on *S. pneumoniae* isolates with levofloxacin MICs of ≥1.5 µg/ml with a battery of fluoroquinolones.

Materials and Methods

- Isolates were collected between January 1, 2000, and December 31, 2001, from 124 geographically distributed centers in the USA and one center in Canada.
- Each center was required to collect a total of 75 clinically significant isolates of *S. pneumoniae*.
- All isolates in this publication were derived from patients ≥16 years of age and were clinical specimens of the blood, cerebrospinal fluid or other sterile body sites. Only one isolate per patient was accepted.
- Organism collection, transport, confirmation of organism identification, as well as construction and management of a centralized database, were coordinated by International Health Management Associates, Inc. (Rolling Meadows, IL, USA).

Antimicrobial Susceptibility Testing

- Each isolate of *S. pneumoniae* was tested in phase I.
- Phase I: primary screening was performed against erythromycin, clindamycin, gemifloxacin and levofloxacin by disk diffusion. Each isolate was also tested against penicillin, gemifloxacin and levofloxacin by the concentration gradient agar diffusion method (Etest).
- Phase II: a panel of quinolones was tested against any isolates from phase I that had a levofloxacin Etest MIC value of ≥1.5 µg/ml and/or gemifloxacin zone of ≥15 mm or Etest MIC value of ≥0.25 µg/ml. Phase II included testing against gemifloxacin, moxifloxacin, gatifloxacin and levofloxacin by Etest methodology.
- Etest methodology followed manufacturer's guidelines (AB Biodisk, Solna, Sweden). Disk diffusion methodology proceeded according to NCCLS[2] and manufacturer's guidelines (Becton-Dickinson, Sparks, MD, USA).
- Quality control of Etest and antimicrobial disks was performed following NCCLS[2] and manufacturer's guidelines.

Results

Results of the study are shown in Tables 1–4.

Table 1. Source and Distribution (n) of 2517 *S. pneumoniae* Isolates from Sterile Body Sites

Source	n	% of total
Blood	2427	96.4
Cerebrospinal fluid	10	0.4
Pleural	41	1.6
Thoracentesis	18	0.7
Paracentesis	21	0.9
Total	2517	100

Table 2. Phase I *In Vitro* Activity and Susceptibility of Gemifloxacin and Comparators Against 2517 *S. pneumoniae* Isolates from Sterile Body Sites

Antimicrobial	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	%S	%I	%R
Gemifloxacin[a]	0.032	0.047	99.6	0.3	0.1
Levofloxacin	0.75	1	99.4	0.2	0.4
Penicillin	0.032	2	70.5	13.7	15.8
Erythromycin[b]	–	–	77.3	1.2	21.5
Clindamycin[b]	–	–	94.0	1.4	4.6

%S, percentage susceptible; %I, percentage intermediate; %R, percentage resistant
[a]Analysis for gemifloxacin was based on susceptible, intermediate and resistant breakpoints of ≤0.25, 0.5 and ≥1.0 µg/ml, respectively
[b]Disk diffusion

Discussion

Fluoroquinolone activity against respiratory strains of *S. pneumoniae* has been documented in several large surveillance studies.[3-7] The MIC_{90}s of gemifloxacin, moxifloxacin, gatifloxacin and levofloxacin are reported as 0.03, 0.25, 0.5 and 1 µg/ml, respectively, for respiratory strains.[4-6] We evaluated current isolates of *S. pneumoniae* from sterile body sites, such as blood and cerebrospinal fluid, as strains from these locations are associated with mortality rates as high as 40–55% and relatively few susceptibility data exist on isolates from these sources.[2]

Approximately 96% of the isolates collected from sterile body sites in this study came from blood specimens with the remaining 4% split between cerebrospinal fluid, pleural, thoracentesis and paracentesis sources (Table 1). Of the 2517 isolates, 70.5% were susceptible to penicillin, with a penicillin resistance rate of 15.8%, similar to that seen in respiratory isolates in the USA (Table 2). The penicillin non-susceptibility rate (>0.06 µg/ml) of 29.5% was somewhat lower than the 35–47% being reported in respiratory isolates or groups of *S. pneumoniae* from unspecified sources.[8-11] It must be noted that the role that drug-resistant *S. pneumoniae* strains play in patient outcome is unclear. Of the isolates from sterile body sites, over 99% were susceptible to the study quinolones gemifloxacin and levofloxacin. Gemifloxacin, at 0.047 µg/ml, had a lower MIC_{90} than levofloxacin, at 1.0 µg/ml. Resistance rates for gemifloxacin and levofloxacin were 0.1% (2 isolates) and 0.4% (10 isolates), respectively. The resistance rates for levofloxacin were similar to the 0.6% found by Chen *et al.*[12] (Canada 1999) and Sahm *et al.*[13] (USA 1999) in *S. pneumoniae* isolates from respiratory, blood and various other sources.

One of the objectives of this study was to examine the resistance patterns of the newer fluoroquinolones in isolates with elevated levofloxacin MICs (≥1.5 µg/ml). These isolates are associated with first- and/or second-step mutations and may be used as a screen for quinolone resistance development in *S. pneumoniae*.[12] There were 137 (5.4%) isolates with elevated levofloxacin MICs from the total of 2517 sterile body site isolates. Of these, gemifloxacin's MIC_{90} (0.125 µg/ml) was 24-fold more potent than levofloxacin (3 µg/ml) and 6-fold more potent than gatifloxacin or moxifloxacin (0.75 µg/ml for both) (Tables 3 and 4). Gemifloxacin's and moxifloxacin's modal MICs were both 2 doubling dilutions below their respective susceptible breakpoints (Figure 1). Levofloxacin and gatifloxacin were 0.5 and 1 doubling dilutions below their respective breakpoints. There were no phase II isolates with gemifloxacin MICs > 32 µg/ml compared with moxifloxacin with 2 (1.5%), gatifloxacin with 3 (2.2%) and levofloxacin with 9 (6.7%) isolates with MICs > 32 µg/ml.

Gemifloxacin exhibited the lowest MIC_{90} against all *S. pneumoniae* sterile body site isolates of all the study drugs tested. Gemifloxacin also had the lowest MIC_{90} compared with all study drugs for isolates with elevated levofloxacin MICs, i.e. against those isolates with presumed first- and/or second-step mutations. These data suggest that gemifloxacin has similar activity to *S. pneumoniae* strains isolated from sterile body sites as those isolated from respiratory sources.

Table 3. Frequency Distribution (n) and Cumulative Percentage Inhibited (Cum%) of 137 Phase II Isolates of *S. pneumoniae* with Levofloxacin MICs of ≥1.5 µg/ml at Each MIC Tested

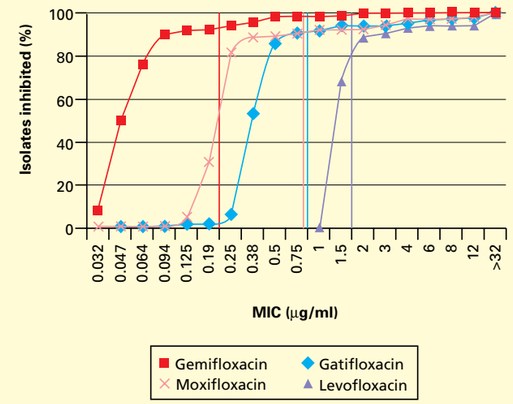
	MIC (µg/ml)																		
	0.032	0.047	0.064	0.094	0.125	0.19	0.25	0.38	0.5	0.75	1	1.5	2	3	4	6	8	12	>32
Gemifloxacin, n	11	57	36	19	2	1	2	3	4				1	1					
Cum%	8.0	49.6	75.9	89.8	91.2	92.0	93.4	95.6	98.5				99.3	100					
Moxifloxacin, n	1			1	6	34	69	10	1	2	2		1	2	4			2	2
Cum%	0.7			1.5	5.8	30.7	81.0	88.3	89.1	90.5	92.0		92.7	94.2	97.1			98.5	100
Gatifloxacin, n		1			1		7	64	44	7	1	3			2	2	1	1	3
Cum%		0.7			1.5		6.6	53.3	85.4	90.5	91.2	93.4			94.9	96.4	97.1	97.8	100
Levofloxacin, n												94	27	3	3	1			9
Cum%												68.6	88.3	90.5	92.7	93.4			100

Table 4. Phase II *In Vitro* Activity and Susceptibility of Gemifloxacin and Comparators Against 137 *S. pneumoniae* Isolates with Levofloxacin MICs of ≥1.5 µg/mlfrom Sterile Body Sites

Antimicrobial	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	%S	%I	%R
Gemifloxacin[a]	0.064	0.125	93.4	5.1	1.5
Moxifloxacin	0.25	0.75	92.0	0.7	7.3
Gatifloxacin	0.38	0.75	91.2	2.2	6.6
Levofloxacin	1.5	3	88.3	4.4	7.3

%S, percentage susceptible; %I, percentage intermediate; %R, percentage resistant
[a]Analysis for gemifloxacin was based on susceptible, intermediate and resistant breakpoints of ≤0.25, 0.5 and ≥1.0 µg/ml, respectively

Figure 1. Cumulative Percentage of Isolates Inhibited at Each MIC Tested for Gemifloxacin and Comparators Against 137 *S. pneumoniae* Isolates with Levofloxacin MICs of ≥1.5 µg/ml from Sterile Body Sites



Vertical lines represent susceptible breakpoints

Conclusions

- Gemifloxacin and levofloxacin had excellent *in vitro* activity, over 99% susceptibility, against all isolates of *S. pneumoniae* from sterile body sites.
- Quinolone resistance (levofloxacin MICs ≥ 8 µg/ml) was 0.4% in *S. pneumoniae* isolated from sterile body sites.
- Gemifloxacin demonstrated the lowest MICs against *S. pneumoniae* from sterile body sites compared with moxifloxacin, gatifloxacin and levofloxacin, including those isolates with potential first- and/or second-step mutations (quinolone-resistant specific).
- Gemifloxacin showed no difference in its activity against *S. pneumoniae* isolated from sterile body sites and respiratory tract sources.
- Increasing multiple-drug-resistant *S. pneumoniae* warrant continuous monitoring nationally and worldwide.

Acknowledgements

We gratefully acknowledge the contributions of all the FACTS study participants: Dr W. Agger, Dr K. Aldridge, Dr D. Amsterdam, Ms M. Ansara, Mr V. Ash, Ms R. Baltzer, Dr E. Baron, Ms G. Bostic, Dr P. Bourbeau, Ms G. Brain, Dr W. Brown, Dr M. Burday, Ms M. Campbell, Dr K. Carroll, Ms B. Chan, Dr K. Chapin, Ms D. Citron, Dr T. Cleary, Dr M. Cole, Dr N. Cornish, Dr J. Daly, Ms B. Davis, Ms S. Davis, Dr G. Denys, Dr M. Desjardins, Dr J. DiPersio, Ms M. Dumont, Dr M. Dykstra, Ms B. Faulkner, Dr M.J. Ferraro, Mr L. Freundlich, Dr T. Fritsche, Ms D. Fuller, Ms C. Garlisi, Ms J. Gibbs, Dr C. Ginocchio, Dr N. Glover, Dr L. Gonzalez III, Ms L. Greacen, Dr N. Habib, Dr G. Hageage, Dr G. Hall, Dr D. Halstead, Dr B. Hanna, Ms B. Hansen, Dr D. Hardy, Dr M. Hayden, Dr K. Hazen, Dr J. Heelan, Ms A. Hermann, Dr A. Herring, Ms J. Hindler, Mr C. Hogan, Dr R. Horvat, Ms J. Houk, Mr J. Hunter, Dr M. Jacobs, Dr S. Jenkins, Dr R. Jerris, Ms P. Jones, Dr R. Jones, Dr A. Junkins, Dr S. Kehl, Ms L. Lane, Dr M. LaRocco, Dr D. Larone, Ms L. Lee, Ms A. Lim, Ms J. Luteran, Dr K. Madaras-Kelly, Dr L. Mann, Dr J. McKitrick, Ms M. Mitchell, Ms J. Monahan, Ms P. Montgomery, Dr M. Mufson, Dr D. Musher, Dr R. Nachum, Mr B. Nethin, Ms C. Norconk, Mr I. Northern, Ms S. Overman, Dr G. Pankey, Dr C. Park, Ms R. Paxson, Dr D. Pombo, Dr K. Rand, Ms D. Reardon, Dr K. Reed, Dr W. Riley, Ms D. Sanchez, Ms C. Sandlin, Dr R. Sautter, Mr R. Scheeler, Dr P. Schreckenberger, Mr D. Schwartz, Ms B. Schwindt, Dr S. Sharp, Dr R. Silberman, Dr J. Snyder, Dr P. Southern Jr, Mr D. Sparlin, Dr C. Spiegel, Dr M. Stanley, Dr J. Steele, Ms L. Steele-Moore, Dr G. Stein, Dr G. Steinkraus, Ms S. Strauss, Mr T. Tay, Ms J. Tetreault, Dr R. Thomson Jr, Mr D. Thornton, Dr K. Tu, Dr R. Van Enk, Dr K. Van Horn, Dr R. Venezia, Ms M. Villanti, Dr K. Waites, Dr A. Wanger, Mr B. Wares, Mr J. Yates.

References

1 Centers for Disease Control. Defining the public health impact of drug-resistant *Streptococcus pneumoniae*: report of a working group. *MMWR* 1996; 45(no. RR-1). http://www.cdc.gov/mmwr/preview/mmwrhtml/00040449.htm
2 National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Disk Susceptibility Tests, 7th edn. Approved Standard M2-A7. Wayne, PA, USA: NCCLS, 2000.
3 Hoban DJ, Bouchillon SK, Karlowsky JA, *et al.* A comparative *in vitro* surveillance study of gemifloxacin activities against 2,632 recent *Streptococcus pneumoniae* isolates from across Europe, North America, and South America. The Gemifloxacin Surveillance Study Research Group. *Antimicrob Agents Chemother* 2000; 44: 3008–3011.
4 McCloskey L, Moore T, Niconovich N, *et al. In vitro* activity of gemifloxacin against a broad range of recent clinical isolates from the USA. *J Antimicrob Chemother* 2000; 45(Suppl. S1): 13–21.
5 Wise R, Andrews JM. The *in-vitro* activity and tentative breakpoint of gemifloxacin, a new fluoroquinolone. *J Antimicrob Chemother* 1999; 44: 679–688.
6 Odland BA, Jones RN, Verhorf J, *et al.* Antimicrobial activity of gatifloxacin (AM-1155, CG5501), and four other fluoroquinolones tested against 2,284 recent clinical strains of *Streptococcus pneumoniae* from Europe, Latin America, Canada and the United States. The SENTRY Antimicrobial Surveillance Group (America and Europe). *Diagn Microbiol Infect Dis* 1999; 34: 315–320.
7 Blondeau JM, Laskowski R, Bjarnason J, Stewart C. Comparative *in vitro* activity of gatifloxacin, grepafloxacin, levofloxacin, moxifloxacin and trovafloxacin against 4151 Gram-negative and Gram-positive organisms. *Int J Antimicrob Agents* 2000; 14: 45–50.
8 Castillo EM, Rickman LS, Brodine SK, *et al. Streptococcus pneumoniae*: bacteremia in an era of penicillin resistance. *Am J Infect Control* 2000; 28: 239–243.
9 Mason EO Jr, Lamberth LB, Kershaw NL, *et al. Streptococcus pneumoniae* in the USA: *in vitro* susceptibility and pharmacodynamic analysis. *J Antimicrob Chemother* 2000; 45: 623–631.
10 Doern GV, Brueggemann AB, Huynh H, Wingert E. Antimicrobial resistance with *Streptococcus pneumoniae* in the United States, 1997–98. *Emerging Infect Dis* 1999; 5: 757–765.
11 Thornsberry C, Jones ME, Hickey ML, *et al.* Resistance surveillance of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* isolated in the United States, 1997–1998. *J Antimicrob Chemother* 1999; 44: 749–759.
12 Chen DK, McGeer A, de Azavedo JC, Low DE. Decreased susceptibility of *Streptococcus pneumoniae* to fluoroquinolones in Canada. *N Engl J Med* 1999; 341: 233–239.
13 Sahm DF, Karlowsky JA, Kelly LJ, *et al.* Need for annual surveillance of antimicrobial resistance in *Streptococcus pneumoniae* in the United States: 2-year longitudinal analysis. *Antimicrob Agents Chemother* 2001; 45: 1037–1042.

Presented at the 102nd General Meeting of the American Society for Microbiology, Salt Lake City, UT, USA. May 19–23, 2002.