Evaluation of Garenoxacin (GRN) Activity Tested Against All Patterns of Multi-Resistant (MDR) *S. pneumoniae*: Multi-Center Studies of Nearly 15,000 Isolates

RN JONES, M STILWELL, HS SADER, TR FRITSCHE, DM JOHNSON

JMI Laboratories, North Liberty, IA, USA

1572

ECCMID 2006

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

Amended Abstract

Objective: To determine the possible co-resistance (R) patterns among contemporary worldwide (1999 - 2005) isolates of *S. pneumoniae* (SPN) and the effect on a new des-F(6) quinolone, garenoxacin (GRN). MDR patterns were defined by CLSI breakpoint criteria (2005) for six agents (penicillin [PEN], cefuroxime axetil [CROX], erythromycin [ER], clindamycin [CL], tetracycline [TC], TMP/SMX [T/S]).

Methods: A total of 14,665 SPN strains were susceptibility (S) tested by CLSI broth microdilution methods, with isolates originally cultured in laboratories in Europe, Asia, Australia, Africa and the Americas. Comparison fluoroquinolones (FQs, 4) were ciprofloxacin, gatifloxacin (GATI), levofloxacin (LEVO) and moxifloxacin (MOXI). All S patterns were determined and then the S rates calculated for non-pattern agents determined in each group having >= 100 occurrences. See table for prevalence of >= 2%.

Results: Fifty-two distinct patterns were detected, of which 10 (81.8% of all strains) predominated. Among MDR SPN strains (R at >=2 agents; 39.6% of isolates), GRN remained very active with 99.88% S at <=1 mg/L (MIC_{90}, 0.06 mg/L). SPN with R to only one drug or a complete S pattern had 99.93% S to GRN (p>0.05). This potency for GRN was greater than GATI (MIC_{90}, 0.5 mg/L), LEVO (MIC_{90}, 1 mg/L) and MOXI (MIC_{90}, 0.25 mg/L), all providing a 99.00% S rate. Patterns with R to four agents had the lowest GRN-S rate at 99.55%. Strains R to all agents were completely GRN-S (see table).

10 Most Common S patterns for SPN

PEN	CROX	ER	CL	TC	T/S	No tested (%)	GRN MIC_{90}	GRN %<= 1mg/L
S	S	S	S	S	S	6,656 (45.4)	0.06	99.9
R	S	S	S	S	S	418 (2.9)	0.06	99.8
S	S	R	S	S	S	336 (2.3)	0.06	100.0
S	S	S	S	R	S	333 (2.3)	0.06	100.0
S	S	S	S	S	R	1,085 (7.4)	0.06	100.0
R	R	S	S	S	R	685 (4.7)	0.06	100.0
S	S	R	R	R	S	293 (2.0)	0.06	100.0
R	R	R	S	S	R	440 (3.0)	0.06	99.3
R	R	R	S	R	R	669 (4.6)	0.06	100.0
R	R	R	R	R	R	1,051 (7.2)	0.06	100.0
-	-	-	-	-	-	14,665 (100.0)	0.06	99.9

Conclusion: GRN was highly active (99.91% at <= 1 mg/L) against all SPN R patterns including MDR phenotypes (5,811 strains). This activity was greater than comparison marketed FQs (4), macrolides, and beta-lactams, but comparable (near 100% S) to glycopeptides, e.g. vancomycin (data not shown). Continued development of GRN for serious MDR SPN infections appears warranted.

Introduction

As rates of resistance to ß-lactams (penicillins, oral/parenteral cephalosporins, ß-lactamase inhibitor combinations) and macrolides (azithromycin, clarithromycin, erythromycin), clindamycin, tetracycline, and trimethoprim/sulfamethoxazole (TMP/SMX) have escalated among *Streptococcus pneumoniae* isolates for community-acquired respiratory tract infections (CA-RTI), the fluoroquinolones have become a viable treatment option, particularly for community-acquired pneumonia (CAP). Recent treatment guidelines promulgated by national societies worldwide have recommended the newer "respiratory" fluoroquinolones (e.g. levofloxacin, gatifloxacin, moxifloxacin, gemifloxacin) for CAP. Increased and, at times, indiscriminate use of fluoroquinolones has resulted in a dramatic increase in fluoroquinolone resistance to both nosocomial and community-acquired pneumococci in the past decade. Pneumococcal resistance as measured by a ciprofloxacin MIC at ≥ 4 mg/L or by levofloxacin non-susceptibility (MIC, ≥ 4 mg/L) have steadily increased to >1% in North America and elsewhere. Therefore, the search for quinolone compounds with greater potency, more favorable pharmacokinetic/pharmacodynamic features and a reduced potential to select for resistant QRDR mutants, has become essential.

Garenoxacin is a novel des-F(6)-quinolone that lacks the C6-position fluorine and has a unique difluoromethoxy substitution at position C8. These alterations resulted in a drug with improved potency against both DNA gyrases and topoisomerase IV. Garenoxacin has been described as highly active against important Gram-positive and –negative pathogens including Enterobacteriaceae, staphylococci, streptococci, *Acinetobacter* spp. and some other Gram-negative non-fermentative bacilli, *Haemophilus influenzae*, *Moraxella catarrhalis*, atypical respiratory tract pathogens, many enterococci and anaerobes. These features are complemented by a favorable pharmacokinetic/pharmacodynamic profile, leading to a high AUC/MIC ratio. Indeed, of existing quinolones, garenoxacin has the highest AUC/MIC ratio for eradicating *S. pneumoniae*. This high AUC/MIC ratio leads to a greater probability of favorable target attainment that has been associated with successful bacterial eradication and minimization of mutational events (low MPC values). These elements of spectrum and potency favor garenoxacin applications to CA-RTI (hospitalized or ambulatory patients).

The in vitro testing results for garenoxacin from the SENTRY Antimicrobial Surveillance Program platform were summarized to assess the spectrum and potency versus *S. pneumoniae* isolated for patients with CA-RTI. A total of 14,665 (1999 - 2005) isolates were analyzed for resistance patterns to eight drugs (vancomycin and linezolid remained active versus all isolates). Clinical and Laboratory Standards Institute (CLSI) methods as described in documents M7-A7 (2006) and M100-S16 (2006) were used throughout.

Materials and Methods

Bacterial strains. The organisms were consecutively collected and processed in central laboratory systems (JMI Laboratories, North Liberty, Iowa, USA; Women's and Children's Hospital, Adelaide, Australia) using common reference test reagents. Isolates were derived from a wide variety of geographic sources (Program Objectives) for diagnosis of community-acquired or nosocomial respiratory tract infections. In this investigation, the isolates were obtained from medical centers in North America (≥ 30 sites in the USA and Canada), Latin America (10 nations), Europe (≥ 30 sites) and the Asia-Pacific region (nine nations plus South Africa). The RTI pathogen studied was only *S. pneumoniae* (14,665 strains) with 9,437 penicillin [PEN]-susceptible, 2,373 PEN-intermediate, 2,855 PEN-resistant isolates. A total of 541 (3.7%) and 4,550 (31.0%) strains were resistant to ciprofloxacin (≥ 4 mg/L) and erythromycin, respectively.

Susceptibility testing methods. All MIC values were generated using broth microdilution methods (CLSI M7-A7) with panels produced by TREK Diagnostics (Cleveland, Ohio, USA). Mueller-Hinton broth was supplemented where indicated with 2-5% lysed horse blood (streptococci) and HTM components (*Haemophilus*). Concurrent quality assurance was maintained via use of CLSI-recommended strains: *Escherichia coli* ATCC 25922 and 35218, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923 and 29213, *H. influenzae* ATCC 49247 and 49766, and *S. pneumoniae* ATCC 49619. All quality control results were within published MIC ranges (M100-S16) for each agent tested. More than 30 antimicrobial agents were processed each year with selected agents (Table 1) compared to garenoxacin in this presentation. A breakpoint for garenoxacin susceptibility and resistance at ≤1/≥4 mg/L was used for comparison purposes.

The analysis was performed using the antibiogram of penicillin, cefuroxime axetil, erythromycin, clindamycin, tetracycline, trimethoprim/sulfamethoxazole, linezolid and vancomycin. The latter two agents were 100% effective in vitro, so only six agents' results contributed to this analysis (see Tables 1 and 2). Resistant and intermediate MIC results by CLSI criteria were considered resistant (R). The minimum sample size displayed by resistant pattern was ≥ 100 occurrences among nearly 15,000 isolates (Table 2).

Results

- Garenoxacin was very active (MIC_{90}, 0.06 mg/L) against *S. pneumoniae* from this worldwide collection of 14,665 strains (1999 - 2005).

Table 1. Activity of garenoxacin tested against 14,665 *S. pneumoniae* from a global surveillance program sorted by their resistance patterns[a].

Resistances (no. tested)	Garenoxacin MIC (mg/L) 50%	90%	Range	% ≤1 mg/L
None (6,656)	0.06	0.06	≤0.03-4	99.92
One drug (2,198)	0.06	0.06	≤0.03-2	99.95
Multidrug				
Two drugs (1,133)	0.06	0.06	≤0.03-2	99.91
Three drugs (1,357)	0.06	0.06	≤0.03-1	100.00
Four drugs (1,108)	0.06	0.06	≤0.03->4	99.54
Five drugs (1,162)	0.06	0.06	≤0.03-2	99.91
All strains (14,665)	0.06	0.06	≤0.03->4	99.91

a. Resistance to penicillin, cefuroxime, erythromycin, clindamycin, tetracycline or trimethoprim/sulfamethoxazole using CLSI categorical criteria (M100-S16, 2006) from the SENTRY Antimicrobial Surveillance Program (1999 - 2005).

- More than 99% of *S. pneumoniae*, regardless of resistance pattern or mutant genotype (Tables 1 and 2), were inhibited by garenoxacin at ≤ 1 mg/L.
- Analysis of the most prevalent resistance patterns (Table 2) demonstrated the consistent in vitro potency of garenoxacin with complete (100.0% susceptible) coverage of the multidrug-resistant pattern (six antimicrobial classes).
- Table 3 lists the cumulative percentage of *S. pneumoniae* inhibited by six tested fluoroquinolones. Gemifloxacin (MIC_{50}, 0.016 mg/L) was more active than garenoxacin (MIC_{90}, 0.06 mg/L), but at CLSI (gemifloxacin, ≤ 0.12 mg/L) or proposed (garenoxacin, ≤ 1 mg/L) breakpoints, garenoxacin had a superior spectrum of antimicrobial coverage (>99.9 versus 99.3%).

Table 2 . Garenoxacin activity against *S. pneumoniae* having the most common susceptibility/resistance patterns and a minimum of 100 occurrences in the SENTRY Antimicrobial Surveillance Program (1999 - 2005).

Susceptibility (S)/Resistance (R) patterns[a] PEN	CROX	ER	CL	TC	T/S	No. Tested (%)	Garenoxacin MIC_{90}	Garenoxacin % ≤1 mg/L[b]
S	S	S	S	S	S	6,656 (45.4)	0.06	99.92
R	S	S	S	S	S	418 (2.9)	0.06	99.76
S	S	R	S	S	S	336 (2.3)	0.06	100.00
S	S	S	S	R	S	333 (2.3)	0.06	100.00
S	S	S	S	S	R	1,085 (7.4)	0.06	100.00
R	R	S	S	S	S	239 (1.6)	0.06	100.00
R	S	R	S	S	S	120 (0.8)	0.06	99.17
R	S	S	S	S	R	279 (1.9)	0.06	100.00
S	S	R	S	R	S	116 (0.8)	0.06	100.00
S	S	R	S	S	R	131 (0.9)	0.06	100.00
S	S	S	S	R	R	137 (0.9)	0.06	100.00
R	R	S	S	S	R	685 (4.7)	0.06	100.00
R	S	R	S	S	R	100 (0.7)	0.06	100.00
S	S	R	R	R	S	293 (2.0)	0.06	100.00
R	R	R	S	R	S	113 (0.8)	0.06	100.00
R	R	R	S	S	R	440 (3.0)	0.06	99.32
R	R	S	S	R	R	110 (0.8)	0.06	100.00
R	S	R	R	R	S	182 (1.2)	0.06	99.45
S	S	R	R	R	R	164 (1.1)	0.06	99.39
R	R	R	R	S	R	196 (1.3)	0.06	100.00
R	R	R	R	S	R	140 (1.0)	0.06	100.00
R	R	R	S	R	R	669 (4.6)	0.06	100.00
R	S	R	R	R	R	157 (1.1)	0.06	99.36
R	R	R	R	R	R	1,051 (7.2)	0.06	100.00

a. Intermediate and resistant MIC results were defined as resistant (R). PEN = penicillin, CROX = cefuroxime axetil, ER = erythromycin, CL = clindamycin, TC = tetracycline, and T/S = trimethoprim/sulfamethoxazole.
b. Range of inhibition was 99.17 to 100.00% at ≤ 1 mg/L.

Table 3. Comparisons of five fluoroquinolones and garenoxacin tested against the 14,665 *S. pneumoniae* in the SENTRY Program (1999 - 2005).

Fluoroquinolone	MIC (mg/L) 50%	90%	Cum. % inhibited at: ≤0.12	0.25	0.5	1	2
Gemifloxacin	0.016	0.06	99.3[a]	99.8	99.9	>99.9	>99.9
Garenoxacin	0.06	0.06	98.9	99.1	99.5	>99.9	>99.9
Moxifloxacin	0.12	0.25	86.2	98.7	99.0	99.1	99.6
Gatifloxacin	0.25	0.5	-[b]	75.5	98.7	99.0	99.1
Levofloxacin	1	1	-	-	28.6	97.3	99.0
Ciprofloxacin	1	2	0.2	0.9	11.1	70.0	96.3

a. Underline value is the percentage of strains inhibited at the CLSI breakpoint for susceptibility.
b. - = not tested

Conclusions

- Garenoxacin demonstrated the widest antimicrobial coverage of *S. pneumoniae* isolates from six continents, spanning a time frame from 1999 to 2005, when compared to other fluoroquinolones.
- Garenoxacin also inhibited >99.9% of multidrug-resistant phenotypes, many that were resistant to 3 - 6 antimicrobial classes.
- Garenoxacin should be investigated further against these emerging resistant *S. pneumoniae* isolates in both the ambulatory and hospital environment.
- The high AUC/MIC ratio of garenoxacin versus *S. pneumoniae* could position it as a valuable respiratory tract quinolone treatment option.

Selected References

Anderegg TR, Biedenbach DJ, Jones RN (2003). Re-evaluation of quality control guidelines for gatifloxacin and garenoxacin (BMS284756) when susceptibility testing *Haemophilus influenzae* and *Streptococcus pneumoniae*. *Diagn Microbiol Infect Dis* 46: 147-150.

Anderegg TR, Jones RN (2004). Bactericidal activity of garenoxacin tested by kill-curve methodology against wild type and QRDR mutant strains of *Streptococcus pneumoniae*. *Diagn Microbiol Infect Dis* 50: 213-217.

Andes D, Craig WA (2003). Pharmacodynamics of the new des-f(6)-quinolone garenoxacin in a murine thigh infection model. *Antimicrob Agents Chemother* 47: 3935-3941.

Biedenbach DJ, Jones RN, Pfaller MA (2001). Activity of BMS-284756 against 2,681 recent clinical isolates of *Haemophilus influenzae* and *Moraxella catarrhalis*: Report from The SENTRY Antimicrobial Surveillance Program (2000) in Europe, Canada and the United States. *Diagn Microbiol Infect Dis* 39: 245-250.

Clinical and Laboratory Standards Institute. (2006). Approved Standard M7-A7: *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th ed*. Wayne, PA: CLSI.

Clinical and Laboratory Standards Institute. (2006). *Performance standards for antimicrobial susceptibility testing, 16th informational supplement M100-S16*. Wayne, PA: CLSI.

Fung-Tomc JC, Minassian B, Kolek B, Huczko E, Aleksunes L, Stickle T, Washo T, Gradelski E, Valera L, Bonner DP (2000). Antibacterial spectrum of a novel des-fluoro(6) quinolone, BMS-284756. *Antimicrob Agents Chemother* 44: 3351-3356.

Ince D, Zhang X, Silver LC, Hooper DC (2002). Dual targeting of DNA gyrase and topoisomerase IV: Target interactions of garenoxacin (BMS-284756, T-3811ME), a new desfluoroquinolone. *Antimicrob Agents Chemother* 46: 3370-3380.

Jones RN, Pfaller MA, Stilwell M (2001). Activity and spectrum of BMS-284756, a new des-F (6) quinolone, tested against strains of ciprofloxacin-resistant Gram-positive cocci. *Diagn Microbiol Infect Dis* 39: 133-135.

Jones RN, Biedenbach DJ (2003). Comparative activity of garenoxacin (BMS-284756), a novel desfluoroquinolone, tested against 8,331 isolates from community-acquired respiratory tract infections: North American results from the SENTRY Antimicrobial Surveillance Program (1999-2001). *Diagn Microbiol Infect Dis* 45: 273-278.

Lister PD (2003). Impact of AUC/MIC ratios on the pharmacodynamics of the des-F(6) quinolone garenoxacin (BMS-284756) is similar to other fluoroquinolones. *J Antimicrob Chemother* 51: 199-202.

Lynch III, JP, Zhanel GG (2005). Escalation of antimicrobial resistance among *Streptococcus pneumoniae*: implications for therapy. *Sem Resp Crit Care Med* 26:575-616.

Morosini MI, Loza E, del Campo R, Almaraz F, Baquero F, Canton R (2003). Fluoroquinolone-resistant *Streptococcus pneumoniae* in Spain: Activities of garenoxacin against clinical isolates including strains with altered topoisomerases. *Antimicrob Agents Ch*emother 47: 2692-2695.

Pankuch GA, Nagai K, Davies TA, Jacobs MR, Appelbaum PC (2002). Antipneumococcal activity of BMS-284756 compared to those of six other agents. *Antimicrob Agents Chemother* 46: 251-254.

Saravolatz L, Manzor O, Pawlak J, Belian B (2001). Antimicrobial activity of BMS 284756, a novel des-fluoro (6) quinolone and seven fluoroquinolones against *Streptococcus pneumoniae*. Clin Microbiol Infect 7: 572-573.

Schmitz FJ, Milatovic D, Boos M, Mayer S, Fluit AC (2002). In vitro activity of the novel des-F(6) quinolone BMS-284756 against genetically characterized clinical streptococcal isolates, including isolates with reduced quinolone susceptibility. *J Antimicrob Chemother* 49: 698-701.

Takahata M, Mitsuyama J, Yamashiro Y, Yonezawa M, Araki H, Todo Y, Minami S, Watanabe Y, Narita H (1999). In vitro and in vivo antimicrobial activities of T-3811ME, a novel des-F(6)-quinolone. *Antimicrob Agents Chemother* 43: 1077-1084.

Van Wart S, Phillips L, Ludwig EA, Russo R, Gajjar DA, Bello A, Ambrose PG, Costanzo C, Grasela TH, Echols R, Grasela DM (2004). Population pharmacokinetics and pharmacodynamics of garenoxacin in patients with community-acquired respiratory tract infections. *Antimicrob Agents Chemother* 48: 4766-4777.

Weller TM, Andrews JM, Jevons G, Wise R (2002). The in vitro activity of BMS-284756, a new des-fluorinated quinolone. *J Antimicrob Chemother* 49: 177-184.