Garenoxacin Activity Against Isolates from Patients Hospitalized with Community-Acquired Pneumonia (*Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*; 3,087 Strains): Report from the SENTRY Antimicrobial Surveillance Program (1999-2003)

RN JONES, TR FRITSCHE, HS SADER, MG STILWELL
JMI Laboratories, North Liberty, IA, USA

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ECCMID 2005

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

Abstract

Objective: To evaluate the potency of garenoxacin (GRN), a new des-F(6)-quinolone, susceptibility (S) when tested against organisms isolated from patients hospitalized with community-acquired respiratory tract infections (CARTI) i.e. *S. pneumoniae* (SPN), *H. influenzae* (HI) and *M. catarrhalis* (MCAT). Organisms from 1999-2003 were available for study, each isolated in Europe, the Americas or Asia; and their origin was lower respiratory tract cultures. Although, MCAT was monitored, it was not isolated in sufficient numbers for analysis.
Methods: Consecutive, non-duplicate cultures of SPN (1,444) and HI (1,643) were tested by reference NCCLS broth microdilution methods with concurrent QC, and interpretation guided by NCCLS M100-S15 (2005). Comparison agents numbered >20 and included four fluoroquinolones (FQs): ciprofloxacin (CIPRO), gatifloxacin (GATI), levofloxacin (LEVO) and moxifloxacin (MOXI). The QRDR regions of *gyrA, parC* and *parE* were sequenced in those strains with elevated GRN MICs (≥ 0.25 mg/L) or resistant (R) to other FQs.
Results: The SPN collection was characterized as follows: penicillin-R at 70.1% (R rate, 16.6%); macrolide-R at 26.1%; clindamycin-R at 11.9%; and amoxicillin/clavulanate- and ceftriaxone (CTRI)-R at 1.4 and 1.1%, respectively. Rank order of FQ activity was (MIC_{90}, mg/L): GRN (0.06) > MOXI (0.12) > GATI (0.5) > LEVO (1, 0.8% R) > CIPRO (2). The HI collection was defined as follows: ampicillin (AMP)-R at 20.3%; beta-lactamase-negative AMP-R at 1.5% (nearly 80% of strains from Japan; rate was <0.5% for all other regions); GRN $MIC_{50/90}$ at ≤ 0.008/0.016 mg/L; and one FQ-R strain had multiple QRDR mutations. See table for co-R analysis among SPN:

	Penicillin category		
Parameter (1,444 strains)	S	Intermediate	R
GRN			
MIC_{50} (mg/L)	0.06	0.06	0.06
MIC_{90} (mg/L)	0.06	0.06	0.06
% ≤ 1 mg/L	100.0	100.0	99.6
CIPRO MIC ≥ 4 mg/L (%)	3.9	4.7	3.3
LEVO-R (%)	0.6	0.7	1.6
CTRI-R (%)	0.1	0.0	6.2

Co-R trends were observed among beta-lactams, macrolides, tetracyclines, TMP/SMX, but not for FQs. An increase in LEVO-R among penicillin-R SPN was observed. Sixty-one percent of all SPN GRN MIC values were 0.06 mg/L, a dominant MIC mode.
Conclusions: GRN exhibited superior activity (lower MIC results) against SPN and HI isolates associated with pneumonias in CARTI patients; 2- to 32-fold more potent than other FQs. SPN and HI isolates with GRN MIC values of more than 0.25 mg/L were very rare (11/3,087; 0.4%) and secondary to numerous QRDR mutations in *gyrA, parC* and *parE*. GRN appears to be an excellent candidate to treat multi-drug R CARTI pathogens in hospitalized patient populations.

Introduction

As rates of resistance to ß-lactams (penicillins, oral/parenteral cephalosporins, ß-lactamase inhibitor combinations) and macrolides (azithromycin, clarithromycin, erythromycin) escalate among *Streptococcus pneumoniae* isolates from community-acquired respiratory tract infections (CA-RTI), the fluoroquinolones have become the drug of choice, particularly for community-acquired pneumonia (CAP). Treatment guidelines promulgated by national societies worldwide have placed the fluoroquinolones as viable treatment options for CAP. As these agents (levofloxacin, gatifloxacin, gemifloxacin, moxifloxacin) have been used at a greater volume, resistances in pneumococci 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. Therefore, the search for quinolone compounds with greater potency, more favorable pharmacokinetic/pharmacodynamic features and a lower potential to select for resistant QRDR mutants, remains essential.

Garenoxacin (formerly T-3811ME or BMS-284756) 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 (*S. pneumoniae*, viridans group species, and ß-haemolytic streptococci), *Acinetobacter* spp. and some other Gram-negative non-fermentative bacilli, *Haemophilus influenzae*, *Moraxella catarrhalis*, atypical respiratory tract pathogens (Mycoplasmas, *C. pneumoniae*, and *Legionella* spp.), many enterococci and anaerobes, especially Gram-positive species. These features are complimented by the high probability of favorable target attainment (AUC/MIC) that has been associated with successful bacterial eradication and minimization of mutational events among indicated species (i.e. low MPC values). These elements of spectrum and potency favor GRN applications for 1) community-acquired respiratory tract infections (CA-RTI; hospitalized or ambulatory patients); 2) skin and soft tissue infections (complicated with mixed flora or uncomplicated); and 3) selected community-acquired intra-abdominal infection indications.

The in vitro testing results for garenoxacin from the SENTRY Antimicrobial Surveillance Program were summarized from 1999 onward to assess the spectrum and potency versus organisms isolated from patients hospitalized with CA-RTI. A total of 3,087 isolates, composed of two dominant species, *S. pneumoniae* and *H. influenzae*, were analyzed from results generated by the reference (National Committee for Clinical Laboratory Standards [NCCLS], currently the Clinical Laboratory Standards Institute [CLSI]) methods as described in document M7-A6 [2003].

Materials and Methods

Susceptibility testing. All MIC values were generated using broth microdilution methods (CLSI/NCCLS, M6-A7) with panels produced by TREK Diagnostics (Cleveland, Ohio, USA). Mueller-Hinton broth was supplemented where indicated with 2 - 5% lysed horse blood (fastidious species including streptococci) and HTM components (*Haemophilus* species). Concurrent quality assurance was maintained via use of CLSI/NCCLS-recommended strains: *E. coli* ATCC 25922 and 35218; *P. aeruginosa* ATCC 27583; *E. faecalis* ATCC 29212; *S. 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 (CLSI/NCCLS, M100-S15). Approximately 35 - 40 different antimicrobial agents were processed each year with selected agents compared to GRN in this presentation. A susceptible breakpoint for garenoxacin at ≤ 2 mg/L was used for comparison purposes only.

Bacterial strains. The organisms were consecutively collected isolates processed in a central laboratory system (JMI Laboratories, North Liberty, Iowa, USA; Women's and Children's Hospital, Adelaide, Australia; Utrecht University, Utrecht, The Netherlands) using common reference tests. Isolates were derived from a wide variety of clinical sources (Program Objectives) such as: bloodstream (BSI), community-acquired or nosocomial respiratory tract sites (RTI), skin and soft tissue infections (SSTI), urinary tract infections (UTI) and selected other patient populations. In this investigation, the isolates were obtained from hospitalized patients with CA-RTI in medical centers in North America (≥ 30 sites in the USA and Canada), Latin America (10 sites), Europe (≥ 30 sites) and the Asia-Pacific region (nine nations plus South Africa). The distribution of tested species was: *S. pneumoniae* (1,444 including 1,012 penicillin-susceptible [70.1%], 192 penicillin-intermediate [13.3%] and 240 penicillin-resistant [16.6%]), and *H. influenzae* (1,643; 20.3% or 334 strains were ß-lactamase-positive and resistant to ampicillin). Among the ß-lactamase test-negative *H. influenzae*, 24 ampicillin-resistant strains (BLNAR) were identified; 14 isolates from Japan (93.3%). The overall BLNAR rate of occurrence was 0.9%, but only 0.1% for the rest of the world. Garenoxacin MIC values for all BLNAR strains were ≤ 0.03 mg/L, however six strains had ciprofloxacin MIC results at 0.25 or 0.5 mg/L suggesting the presence of first-step QRDR mutations. *Moraxella catarrhalis* strains associated with CAP were rare and are not tabulated here.

Molecular methods. The QRDR was assessed for mutations in *gyrA* or *gyrB* and *parC* or *parE* by PCR application and sequence analyses. *S. pneumoniae* and *H. influenzae* isolates with elevated garenoxacin MIC results (1 - > 4 mg/L) were processed to detect mutations. Only 17 *S. pneumoniae* strains (1.2%) were identified with a garenoxacin MIC at ≥ 0.25 mg/L, and only one isolate of *H. influenzae* was resistant to fluoroquinolones by current CLSI/NCCLS breakpoint criteria (2005).

Results

- The frequency of occurrence for *H. influenzae*, as a cause of CAP, was slightly greater than *S. pneumoniae* (53.2% versus 46.8%, respectively).
- Characteristics of the *S. pneumoniae* isolate population were: penicillin resistance at 16.6% (MIC, ≥ 2 mg/L), macrolide resistance at 26.1% (approximately one-half constitutive expression), tetracycline resistance at 17.3% and trimethoprim/sulfamethoxazole resistance at 18.1%. Nearly 4% of strains had a ciprofloxacin MIC at ≥ 4 mg/L, e.g. possible QRDR mutations.
- The *H. influenzae* isolates were highly susceptible to all of the tested agents except trimethoprim/sulfamethoxazole (80.6% susceptible). BLNAR isolates occurred at a rate of 0.1%, excluding highly endemic Japanese strains.
- Garenoxacin was very active against *S. pneumoniae* (Tables 1 and 2) with all MIC values at ≤ 2 mg/L. Resistances among pneumococci to other classes of antimicrobials did not significantly impact the garenoxacin MIC results (except ciprofloxacin; data not shown; Table 2).
- Rare strains of *S. pneumoniae* (10; 0.7%) and *H. influenzae* (1; 0.06%) had garenoxacin MIC values of ≥ 0.5 mg/L.
- Resistance to penicillin (Table 3) adversely influenced the spectrum of parenteral cephalosporins (ceftriaxone and cefepime), macrolides (erythromycin), tetracyclines (doxycycline) and trimethoprim/sulfamethoxazole. Levofloxacin resistance appeared to be similarly related to penicillin susceptibility, although unrelated to ciprofloxacin resistance results (QRDR mutations). Strains with higher garenoxacin MIC values were most likely to occur among penicillin- and erythromycin-resistant isolates (Table 2).
- Garenoxacin was the most potent quinolone tested (MIC_{90}, 0.06 mg/L), inhibiting all *S. pneumoniae* at ≤ 2 mg/L compared to ≤ 99.3% for the comparator fluoroquinolones (3). Interestingly, 60.9% of all garenoxacin MIC results were 0.06 mg/L.

Table 1. Comparative activity of garenoxacin and selected agents (14) tested against *S. pneumoniae* (1,444 strains) and *H. influenzae* (1,643 strains) isolated from patients hospitalized with CA-RTI (1999 - 2003).

	MIC (mg/L)			% by category:[a]	
Organism/antimicrobial agent (no. tested)	Range	50%	90%	Susceptible	Resistant
S. pneumoniae (1,444)					
Garenoxacin	≤0.03-2	0.06	0.06	100.0[b]	0.0
Ciprofloxacin	≤0.25->2	1	2	-	(3.9)[c]
Gatifloxacin	≤0.03->4	0.25	0.5	99.3	0.6
Gemifloxacin	≤0.12-0.5	≤0.12	≤0.12	99.3	0.1
Levofloxacin	0.12->4	1	1	99.2	0.6
Penicillin	≤0.016-8	≤0.016	2	70.1	16.6
Amoxicillin/Clavulanate	≤2-16	≤2	≤2	96.7	1.4
Ceftriaxone	≤0.25-8	≤0.25	1	95.8	1.1
Erythromycin	≤0.06->8	≤0.06	>8	73.5	26.1
Clindamycin	≤0.06->8	≤0.06	>8	87.4	12.3
Quinupristin/Dalfopristin	≤0.06-2	0.5	0.5	99.8	0.0
Doxycycline	≤0.5->4	≤0.5	>4	76.9	17.3
Linezolid	≤0.25-2	1	1	100.0	-
Trimethoprim/Sulfamethoxazole	≤0.5->2	≤0.5	>2	68.3	18.1
Vancomycin	≤0.12-1	0.25	0.5	100.0	-
H. influenzae (1,643)					
Garenoxacin	≤0.03-8	≤0.03	≤0.03	>99.9	<0.1[d]
Ciprofloxacin	≤0.25->4	≤0.25	≤0.25	>99.9	<0.1[d]
Gatifloxacin	≤0.03->4	≤0.03	≤0.03	>99.9	<0.1[d]
Gemifloxacin	≤0.12->1	≤0.12	≤0.12	>99.9	<0.1[d]
Levofloxacin	≤0.03->4	≤0.03	≤0.03	>99.9	<0.1[d]
Ampicillin	≤0.12->16	0.5	>16	79.7	20.3[e]
Amoxicillin/Clavulanate	≤2-16	≤2	≤2	99.3	0.7
Ceftriaxone	≤0.25-2	≤0.25	≤0.25	100.0	-
Cefuroxime axetil	≤0.12->16	1	2	97.6	1.1
Tetracycline	≤4->8	≤4	≤4	98.8	0.6
Trimethoprim/Sulfamethoxazole	≤0.5->2	≤0.5	>2	80.6	13.7

a. Susceptibility criteria of the CLSI/NCCLS (2005), where available.
b. Garenoxacin susceptibility was defined as ≤ 2 mg/L for comparison purposes only.
c. Resistance criteria of Chen et al. (1999) to indicate frequency of QRDR mutations.
d. One resistant strain with QRDR mutations (multiple).
e. Resistance determined by the detection of a ß-lactamase (nitrocefin).

Table 2. In vitro activity of garenoxacin tested against 1,444 strains of *S. pneumoniae* isolated from hospitalized patients with CA-RTI.

	MIC (mg/L)			Cum. % inhibited at MIC (mg/L)						
Organism group (no. tested)	Range	50%	90%	≤0.03	0.06	0.12	0.25	0.5	1	2
All strains (1,444)	≤0.03-2	0.06	0.06	31.5	92.5	98.8	99.3	99.5	>99.9	100.0
Penicillin-susceptible (1,012)	≤0.03-1	0.06	0.06	29.6	91.5	99.1	99.6	99.6	100.0	-
Penicillin-intermediate (192)	≤0.03-1	0.06	0.06	34.9	94.3	97.4	97.9	99.5	100.0	-
Penicillin-resistant (240)	≤0.03-2	0.06	0.06	37.1	95.0	98.3	99.2	99.2	99.6	100.0
Erythromycin-susceptible (1,061)	≤0.03-1	0.06	0.06	28.9	93.0	99.4	99.8	99.8	100.0	-
Erythromycin-intermediate (6)	≤0.03-0.12	≤0.03	-	66.7	83.3	100.0	-	-	-	-
Erythromycin-resistant (377)	≤0.03-2	0.06	0.06	38.2	91.0	96.8	97.9	98.7	99.7	100.0

Table 3. Variations in garenoxacin MIC results and selected comparison agents among *S. pneumoniae* having various susceptibilities to penicillin.

	Penicillin category		
Antimicrobial agent	Susceptible	Intermediate	Resistant
Garenoxacin			
MIC_{50} (mg/L)	0.06	0.06	0.06
MIC_{90} (mg/L)	0.06	0.06	0.06
% ≤ 1 mg/L	100.0	100.0	99.6
Ciprofloxacin			
% ≥ 4 mg/L[a]	3.9	4.7	3.3
Levofloxacin			
% non-susceptibility	0.6	0.7	1.6
Ceftriaxone/Cefepime			
% resistance	0.1/0.1	0.0/0.0	6.2/1.2
Erythromycin			
% resistant	9.8	53.1	73.3
Doxycycline			
% resistance[b]	8.3	29.2	45.4
Trimethoprim/Sulfamethoxazole			
% resistance	11.1	41.7	82.9

a. Chen et al., 1999.
b. Using breakpoint criteria for tetracycline (CLSI/NCCLS, 2005).

Conclusions

- Against CAP isolates of *S. pneumoniae* and *H. influenzae*, garenoxacin was highly active (MIC_{90}s, ≤ 0.03 or 0.06 mg/L) and represents a potent therapeutic option. This activity was 16-fold greater than levofloxacin.
- Resistances to other antimicrobial classes generally did not adversely impact garenoxacin activity or spectrum.
- The garenoxacin resistance rate among CAP pathogens was rare and significantly less than that observed for other peer drugs/fluoroquinolones tested (gatifloxacin, gemifloxacin, levofloxacin).

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