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Garenoxacin (BMS284756) Activity Against 8,686 S. pneumoniae Including Tentative Susceptibility Testing Criteria

RN Jones, A Mutnick, and D Biedenbach.
The JONES Group/JMI Laboratories, North Liberty, Iowa [www.jmilabs.com]; and Tufts Univ., Boston, MA

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ABSTRACT

Background: Garenoxacin, a novel desfluoroquinolone, has very potent activity against Gram-positive cocci, particularly *S. pneumoniae* (SPN) and other streptococci. This activity compares favorably to those quinolones having FDA antistreptococcal indications and NCCLS SPN susceptibility (S) testing criteria. Garenoxacin was compared to 8 other quinolones and tested by the disk diffusion (DD) method to establish in vitro breakpoint criteria for S and resistance (R).

Methods: All tests (MIC, DD) were performed by NCCLS methods. Twenty antimicrobial agents were tested against 8,686 SPN from Europe, the Asia-Pacific, Latin and North America. This SPN collection had 67.3 and 77.2% S to penicillin (PEN) and erythromycin, respectively. A subset of 668 streptococci (327 SPN) were tested by 5-µg garenoxacin DD and a conservative breakpoint (≤ 2 µg/ml) was proposed with corresponding zone criteria. All QC determinations were within published ranges (NCCLS Minutes, 2002).

Results: Garenoxacin had $MIC_{50/90}$ results at 0.06 µg/ml, 2-fold greater than gemifloxacin (GEM; $MIC_{50/}$ 0.03) and 4-fold less than grepafloxacin (GRE), moxifloxacin (MOX) and trovafloxacin (TRO). Ciprofloxacin and levofloxacin (LEV) MIC_{50} values were 2 and 1 µg/ml, respectively. % S/R were in rank order: garenoxacin (99.9/< 0.1) > GEM (99.8 [≤ 0.25]/0.1) > TRO (99.7/0.3) > gatifloxacin = GRE (99.4/0.5) > LEV (99.3/0.5) > MOX (99.2/0.4). PEN-S and -R strains had the same garenoxacin MIC_{50} and % S (99.9), while LEV % S decreased from 99.6 to 98.2%. Scattergrams comparing garenoxacin MICs and zones for SPN suggest a tentative S zone at ≥ 16 mm and R at ≤ 12 mm. No inter-method serious errors were observed, only 0.3% minor error among nearly 700 streptococci. This breakpoint conforms to published garenoxacin PK/PD parameters by Craig and others.

Conclusions: Garenoxacin was very active against SPN strains worldwide, with a MIC_{90} at 0.06 µg/ml and using the tentative breakpoint (≤ 2 µg/ml) would have a susceptibility rate of > 99.9%. This is the largest SPN collection used to establish potency and S criteria for a quinolone (garenoxacin) prior to NDA filing.

INTRODUCTION

Infections caused by resistant Gram-positive cocci, especially streptococci, are a major problem among community-acquired cases, worldwide. Quinolones are used widely for both empiric and directed oral therapy due to their excellent spectrum and potency enhanced by favorable pharmacokinetics against these strains. Newer advanced generation fluoroquinolone derivatives (gatifloxacin, gemifloxacin [recently withdrawn], moxifloxacin) with expanded activity against Gram-positive pathogens are being introduced in an effort to meet the ever increasing challenge of resistance in the streptococcal species.

Garenoxacin (formerly BMS284756 or T-3811) is a novel desfluoro(6)quinolone. The des-fluoro compounds have been shown to have lower cerebral toxicity in mice. Previous studies have demonstrated comparable in vitro activity to that of gatifloxacin and moxifloxacin, even against anaerobic species. As the newer, more potent quinolones become available for respiratory tract infection therapy, it is important to develop in vitro susceptibility testing criteria for the streptococci and determine their role by high volume in vitro surveillance samples.

This study compares the activity of this new desfluoro(6)quinolone and other selected antimicrobials against the most commonly isolated groups of streptococci (*S. pneumoniae*, viridans group streptococci and ß-haemolytic species) monitored over three years for hospitalized and community-acquired patient infections. The results obtained from disk diffusion were compared to those produced by the reference broth microdilution method described by the National Committee for Clinical Laboratory Standards (NCCLS).

MATERIALS AND METHODS

Organisms tested. A total of 84 medical centers from countries in North America, Latin America, the Asia-Pacific, and Europe submitted isolates for study (SENTRY Antimicrobial Surveillance Program, 1999-2001). The organisms were derived from community-acquire respiratory tract infections. A total of 8,686 *S. pneumoniae* were tested. A subset of 1,160 streptococcal strains were tested: 601 *S. pneumoniae* (60% susceptible to penicillin; ≤ 0.06 µg/ml) and 559 viridans group streptococci and ß-haemolytic streptococci. Several pneumococcal strains were selected for the test development phase that possessed levofloxacin MICs of ≥ 8 µg/ml (resistant and one had an elevated MIC to garenoxacin). Comparative activity of garenoxacin versus eight selected quinolones was also performed.

Susceptibility testing methods. All organisms were tested by reference broth microdilution method and the standardized disk diffusion test. The broth microdilution trays were produced by TREK Diagnostics, Inc. (Westlake, OH) and were validated to be equivalent to NCCLS tests. The garenoxacin 5-µg disks were made by BD Microbiology Systems (Cockeysville, MD). The disk diffusion test results were compared using the proposed susceptibility breakpoint of ≤ 2 µg/ml, although no isolates were identified that would be considered resistant to garenoxacin by previously cited criteria of Fung-Tomc et al. (≤ 4 µg/ml). Linear regression statistics and the determination of potential interpretive errors were used to assess diagnostic accuracy applying M23-A2 criteria.

RESULTS

- The *S. pneumoniae* collection (1999-2001) had the following resistance profile (Table 1):
  - 67.3% susceptibility to penicillin(15.1% high-level resistance)
  - 77.2% susceptibility to erythromycin (56% M-phenotypes)
  - 67.2% susceptibility to trimethoprim/sulfamethoxazole
  - 95.2-96.2% susceptibility to cefepime and ceftriaxone
- Ciprofloxacin resistance at ≥ 4 µg/ml was 2.7% overall and has steadily increased over the three years. All other tested quinolones had resistance rates of ≤ 0.5%, lowest for garenoxacin (0.023%; two strains; Table 1).
- Garenoxacin (MIC_{90}, 0.06 µg/ml) and gemifloxacin (MIC_{90}, ≤ 0.03 µg/ml) were the most potent agents tested against *S. pneumoniae* (Table 1).
- The two strains with reduced susceptibility to garenoxacin (MIC, > 4 and 4 µg/ml) were discovered in Canada (2000) and in the USA (2001). Each strain was susceptible to numerous other agents, but had MICs to all other tested quinolones at > 4 µg/ml (data not shown).
- Co-resistance associated with penicillin resistance was noted for erythromycin and clindamycin, as well as, a trend toward resistance for some quinolones (Table 2). Garenoxacin non-susceptibility was not associated with other resistances.

Table 1. Comparative antimicrobial activity of garenoxacin tested against 8,686 *S. pneumoniae* strains isolated from community-acquired respiratory tract infections in the SENTRY Antimicrobial Surveillance Program (1999-2001)

	MIC (µg/ml)		% by category:	
Antimicrobial agent (no. tested)	50%	90%	Susceptible	Resistant
Garenoxacin (8,686)	0.06	0.06	>99.9[a]	<0.1[a]
Ciprofloxacin	1	2	-	(2.7)[b]
Gatifloxacin	0.25	0.5	99.4	0.5
Gemifloxacin	≤0.03	≤0.03	99.8[c]	<0.1[c]
Grepafloxacin	≤0.12	0.25	99.4	0.4
Levofloxacin	1	1	99.3	0.5
Moxifloxacin	0.12	0.25	99.2	0.4
Sparfloxacin	0.25	0.5	98.4	0.2
Trovafloxacin	0.12	0.25	99.7	0.3
Penicillin	≤0.03	2	67.3	15.1
Erythromycin	≤0.25	8	77.2	21.8
Clindamycin	≤0.25	0.5	89.9	9.8
Ceftriaxone	≤0.25	1	95.2	1.1
Cefepime	≤0.12	1	96.2	0.4
Chloramphenicol	≤2	4	92.0	6.8
Linezolid	1	1	100.0	-
Quinupristin/dalfopristin	0.5	0.5	99.6	<0.1
Trimethoprim/sulfamethoxazole	≤0.5	>2	67.2	22.5
Vancomycin	0.25	0.5	100.0	-

a. More conservative susceptibility criteria at ≤ 1 µg/ml was applied. Only two strains were resistant (≥ 4 µg/ml; 0.023%) to garenoxacin.
b. % resistant by criteria of Chen et al. (1999) at ≥ 4 µg/ml.
c. A breakpoint of ≤ 0.25 µg/ml was utilized based on available PK/PD values.

- Table 3 shows the QRDR mutations (2001 isolates only) encoding resistant-level MICs to ciprofloxacin and levofloxacin. Levofloxacin resistance was associated with common mutations of *gyr A* (S81F or E85K) and *par C* (S79F or K137N or D83Y). The garenoxacin-resistant isolate had double mutations at the following QRDR sites: *gyr A* (S81F, E85K) and *par C* (S79F, D83Y); no mutations were detected in *gyr B* or *par E*.
- Figure 1 illustrates the scattergram of nearly 1,200 streptococci (601 *S. pneumoniae*) tested by garenoxacin MIC and disk diffusion methods. Excellent results were noted with no serious interpretive, inter-method errors and 99.9% absolute agreement. Tentative breakpoints of ≤1 or ≤ 2 µg/ml should be considered with correlate zones of ≥ 16 mm and ≤ 12 mm.

Table 2. Influence of penicillin susceptibility on the activity of six monitored antimicrobial agents tested against 8,686 *S. pneumoniae* strains.

	MIC_{50}/MIC_{90}/% susceptible by penicillin category (no. tested).[a]		
Antimicrobial agent	Susceptible (5,842)	Intermediate (1,349)	Resistant (1,495)
Garenoxacin	0.06/0.06/99.9	0.06/0.06/99.8	0.06/0.06/>99.9
Gatifloxacin	0.25/0.5/99.7	0.25/0.5/99.3	0.25/0.5/98.4
Levofloxacin	1/1/99.6	1/1/99.2	1/1/98.2
Ciprofloxacin	1/2/(2.3)[b]	1/2/(2.7)[b]	1/2/(4.5)[b]
Erythromycin	≤0.25/≤0.25/90.7	≤0.25/>32/61.5	2/>32/35.7
Clindamycin	≤0.25/≤0.25/96.3	≤0.25/>8/81.2	≤0.25/>8/71.2

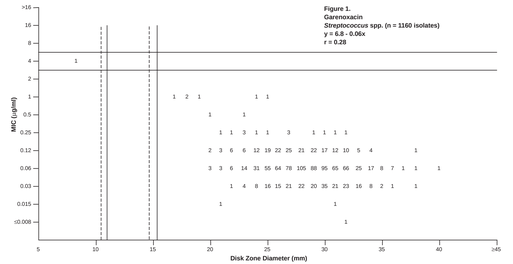
a. Susceptibility criteria of the NCCLS [2002].
b. Percentage in parenthesis indicates the proportion of strains with ciprofloxacin MICs at ≥ 4 µg/ml [Chen et al., 1999].

Table 3. QRDR mutations in selected *S. pneumoniae* isolates associated with elevated ciprofloxacin and levofloxacin MIC values (≥ 4 µg/ml) for SENTRY Antimicrobial Surveillance Program isolates in 2001-2002.

		Mutation of:				
Country (no. tested)	No. of strains	*gyr A*	*gyr B*	*par C*	*par E*	Resistances[a]
USA (20)	3	S81F	-	S79F	-	CL
	1	S81F	-	S79F	I460V	CL
	1	S81F, E85K	-	S79F, D83Y	-	CL
	5	-	-	-	I460V	C
	4	-	-	K137N	I460V	C
	2	-	-	S79Y	-	C
	1	-	-	S79Y	I460V	C
	1	-	-	S52G, K137N	I460V	C
	1	S81F	-	D83N	-	C
	1	-	-	D83N	I460V	C
Canada (16)	6	S81F	-	S79F	-	CL
	1	S81F	-	-	D435N, P454S, I460V	CL
	1	S81F	-	S79F	I460V	CL
	1	E85K	-	S79F	I460V	CL
	1	E85K	-	S79F, K137N	I460V	CL
	3	-	-	S79K	-	C
	3	-	-	S79F, K137N	-	C

a. C = MIC ≥ 4 µg/ml for ciprofloxacin; L = MIC ≥ 4 µg/ml for levofloxacin.

Figure 1. Scattergram for 1,160 streptococci (601 *S. pneumoniae*) tested by NCCLS broth microdilution and disk diffusion methods against garenoxacin. Solid vertical and horizontal lines are tentative MIC breakpoints. Alternative breakpoints are shown with broken lines that may be used for other bacterial species.



CONCLUSIONS

- Garenoxacin, a new desfluoro(6)quinolone (formerly BMS284756), demonstrated very potent activity against 8,686 *S. pneumoniae* isolates from the SENTRY Program isolated from 1999 through 2001.
- Garenoxacin inhibited > 99.9% of pneumococci at ≤ 1 µg/ml and only 0.023% (two strains) of strains were judged to be non-susceptible (MIC, ≥ 4 µg/ml).
- Garenoxacin susceptibility tests by NCCLS methods should be accurate using the criteria proposed in Figure 1.

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