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In Vitro Activities of Garenoxacin (BMS 284756) against 108 Clinical Isolates of *Gardnerella vaginalis*

Ellie J. C. Goldstein,* Diane M. Citron, C. Vreni Merriam, Yumi A. Warren, Kerin L. Tyrrell, and Helen T. Fernandez

R. M. Alden Research Laboratory, Santa Monica-University of California Los Angeles Medical Center, Santa Monica, California 90404, and the University of California Los Angeles School of Medicine, Los Angeles, California 90073

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Garenoxacin (BMS 284756) was active against 105 of 108 (97%) recent clinical *Gardnerella vaginalis* isolates at ≤2 μg/ml by using the reference agar dilution method for anaerobes. Twenty-eight percent of isolates (31 of 108) were resistant to metronidazole, and 44% were resistant to doxycycline. All were susceptible to clindamycin and ampicillin-sulbactam.

Vaginal microbiology reflects a dynamic and complex ecosystem that is still not completely understood. *Gardnerella vaginalis* is a gram-negative to gram-variable, nonmotile, pleomorphic rod that is associated with bacterial vaginosis typically in concert with anaerobes (2, 11). It has been found in ~88% of vaginal samples from women with bacterial vaginosis, but no specific biotype is associated with infection (2). While many treatment options exist for bacterial vaginosis, none are completely efficacious (3, 7) due to the complexity of the perturbation of the vaginal flora in bacterial vaginosis, including the role of local pH on the activity of antimicrobial therapy. A search for a more effective therapy is ongoing (5).

The activity of antimicrobial agents against *G. vaginalis* has shown that in vitro activity does not necessarily correlate with in vivo efficacy in treating the complex flora found in bacterial vaginosis (7, 11). While beta-lactam agents appear to be active against *G. vaginalis* isolates, their clinical efficacy remains limited (5, 7), possibly due to the presence of beta-lactamase-producing anaerobes. Resistance of *G. vaginalis* to metronidazole and associated clinical failures have been reported (3). One report suggests that certain biotypes tend to be more resistant to metronidazole than others (2).

While *G. vaginalis* isolates are usually resistant to nalidixic acid (9), Kato et al. (10) noted that sitafloxacin inhibited *G. vaginalis* at a concentration of 0.1 μg/ml, which suggested that quinolones might have a role in the therapy for bacterial vaginosis. A study from our laboratory (1) has noted that some of the newer fluoroquinolones also have enhanced in vitro activity against anaerobic pathogens from the lower female genital tract. Garenoxacin is a new des-F(6)quinolone that lacks the six-position fluorine characterizing the previous generation of fluoroquinolones. Preliminary data (4, 8) indicate that this drug has a broad spectrum of activity against many gram-positive and gram-negative aerobes and anaerobes, including certain strains that are resistant to other fluoroquinolones. Consequently, we studied the in vitro activity of garenoxacin against recent clinical isolates of *G. vaginalis*.

* Corresponding author. Mailing address: 2021 Santa Monica Blvd., Suite 740 East, Santa Monica, CA 90404. Phone: (310) 315-1511. Fax: (310) 315-3662. E-mail: EJCGMD@aol.com.

Strains were recovered from recent human clinical specimens (during the period from 2001 to 2002) and identified by standard criteria (9, 13). Clinical vaginal specimens were plated onto vaginalis agar (Hardy Diagnostics, Santa Maria, Calif.) and incubated in a 5% CO_2 environment. Small translucent colonies that were beta-hemolytic and catalase negative and which exhibited gram-variable, pleomorphic, coccobacillary morphology upon Gram staining were determined to be *G. vaginalis* (13). Standard laboratory powders were supplied as follows: garenoxacin from Bristol Myers Squibb, Wallingford, Conn.; levofloxacin from Ortho McNeil Pharmaceuticals, Raritan, N.J.; ampicillin-sulbactam from Pfizer Inc., New York, N.Y.; cefoxitin from Merck & Co. Inc., Rahway, N.J.; metronidazole from Searle, Skokie, Ill.; clindamycin from Pharmacia, Kalamazoo, Mich.; and doxycycline from Sigma Chemical Co., St. Louis, Mo.

Frozen cultures were transferred twice onto brucella agar supplemented with vitamin K1, hemin, and sheep blood to ensure purity and good growth. Cell paste from fresh growth of the isolates was transferred into brucella broth to obtain a density equal to the no. 0.5 McFarland standard. The final inoculum contained 10^5 CFU/spot. Susceptibility testing was according to the reference method for anaerobes and utilized brucella agar supplemented with vitamin K1, hemin, and sheep blood (14), since there is no NCCLS-recommended method for *Gardnerella* sp. and many strains grow better in an anaerobic environment. This method has been used in the past with good results (6).

The antimicrobial agents were reconstituted according to the manufacturers' instructions. Serial twofold dilutions were prepared on the day of testing and added to the media to yield various concentrations in accord with breakpoints and achievable levels for each drug class. Agar plates containing antimicrobial agents were inoculated by using a Steers replicator. Control plates without any antimicrobial agents were inoculated before and after each set of antimicrobial agent-containing plates. All incubations were carried out at 35°C in an anaerobic environment, and plates were examined at 48 h. The MIC was defined as the lowest concentration of drug where a marked change occurred in the appearance of growth compared to the appearance of growth on the control plates (14).

TABLE 1. Comparative in vitro activity of garenoxacin (BMS 284756) and six other agents against 108 clinical isolates of *G. vaginalis*

Antimicrobial agent	MIC (μg/ml)[a]			
	Range	50%	90%	ATCC[b]
Garenoxacin	≤0.015–8	0.5	1	0.5
Levofloxacin	0.5–>8	1	1	1
Doxycycline	0.125–32	1	16	0.5
Metronidazole	≤0.25–>32	8	>32	8
Clindamycin	≤0.03–0.25	≤0.03	0.125	≤0.03
Ampicillin-sulbactam	≤0.03–2	0.125	0.25	≤0.03
Cefoxitin	0.06–>16	0.5	4	0.25

[a] MICs at which 50 and 90% of the strains were inhibited, respectively.
[b] Activity (MIC) of agent against *G. vaginalis* ATCC 14018.

Quality control strains included *G. vaginalis* ATCC 14018, *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741, and *Eubacterium lentum* ATCC 43055.

Several authors have studied the in vitro activity of antimicrobial agents against *G. vaginalis* (5, 6, 10, 12, 15). Our results with 108 strains are summarized in Table 1. Garenoxacin was active at ≤2 μg/ml against 97% (105 of 108) of *G. vaginalis* strains. The three stains with garenoxacin MICs of 4 to 8 μg/ml were also resistant to levofloxacin and metronidazole. Garenoxacin was generally two- to fourfold more active than levofloxacin against *G. vaginalis* isolates. Kato et al. (10), using supplemented Columbia agar, noted that all 25 isolates tested were susceptible to ≤1.56 μg of levofloxacin/ml; in contrast, six of our strains required ≥8 μg of levofloxacin/ml for inhibition. Levofloxacin-resistant strains (MIC, ≥8 μg/ml) required between 1 and 8 μg of garenoxacin/ml for inhibition, including two strains that required 4 μg of garenoxacin/ml and one strain that required 8 μg of garenoxacin/ml for inhibition. All our isolates were susceptible to ampicillin-sulbactam and clindamycin. Overall, 44% of strains were resistant to doxycycline (MIC, ≥8 μg/ml), including two of the three strains with high garenoxacin MICs. Four strains required ≥16 μg of cefoxitin/ml for inhibition, and 31 (29%) isolates were resistant to metronidazole (MIC, ≥16 μg/ml). There was no relationship between metronidazole and garenoxacin resistance, but 15 of 31 of the metronidazole resistant isolates were also resistant to doxycycline. In 1993, it was reported (6) that 20% of *G. vaginalis* strains were resistant to metronidazole. This increase from 20 to 29% metronidazole resistance suggests that the susceptibility of *G. vaginalis* should be monitored on a regular basis and considered if clinical treatment failure occurs.

Garenoxacin has excellent in vitro activity against *G. vaginalis*. While it is necessary to perform pharmacokinetic-pharmacodynamic studies before valid clinical conclusions may be drawn, garenoxacin's excellent activity against *G. vaginalis*, coupled with its activity against anaerobes (4, 8), suggests that it deserves evaluation in the therapy of bacterial vaginosis and infections involving *G. vaginalis*.

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