

Comparative Antimicrobial Spectrum and Activity Of BMS 284756 (T-3811; A Desfluoroquinolone) Trend Against Anaerobes and Selected Fastidious Species (Campylobacter, Helicobacter, Legionella), Including Limited *In Vitro* Methods Development

P. Rhomberg, D.M. Johnson, R.N. Jones, and D.J. Biedenbach
University of Iowa, Iowa City, Iowa, USA; and The JONES Group, North Liberty, Iowa, USA.

Ronald N. Jones, M.D.
The JONES Group / JMI Laboratories
345 Beaver Creek Centre, Suite A
North Liberty, Iowa 52317
Phone: 319.665.3370
Fax: 319.665.3371
ronald-jones@jonesgr.com

ABSTRACT

Purpose: To analyze the *in vitro* activity and spectrum of BMS 284756 using agar dilution and Etest (AB BIODISK, Solna, Sweden) methods and compare the results between *in vitro* methods when testing anaerobes and selected fastidious species.

Methods: The activities of BMS 284756, ciprofloxacin, gatifloxacin, levofloxacin, and trovafloxacin were evaluated against *Campylobacter jejuni* (38 strains), *Helicobacter pylori* (21 strains), *Legionella* spp. (66 strains), and 197 anaerobic isolates (76 Gram-positive and 121 Gram-negative). All organisms tested were obtained from various collections at the University of Iowa College of Medicine (Iowa City, Iowa), including isolates from the SENTRY Antimicrobial Surveillance Program (1997-2000). Strains were tested using Etest and the agar dilution (AD) method according to NCCLS recommended procedure.

Results: BMS 284756 (MIC₉₀, 0.008 µg/ml) was 4-fold more active than gatifloxacin or trovafloxacin against *H. pylori* strains. Gatifloxacin and BMS 284756 (MIC₅₀ 0.03 µg/ml) were ≥2-fold more active than levofloxacin against *C. jejuni*, but their spectra were equal (89.4% susceptible strains). A comparison of Etest and reference MICs showed a 90% correlation (± two log₂ dilutions) for *C. jejuni*, with the Etest value usually being lower. Against the *Legionella* spp. all comparison fluoroquinolones (MIC₉₀, 0.25 µg/ml; Etest) were 2-fold more potent than BMS 284756, but all strains were considered susceptible. BMS 284756 and trovafloxacin (MIC₉₀, 1 and 2 µg/ml, respectively) were several-fold more potent than other comparators (MIC₉₀s, ≥16 µg/ml) against *B. fragilis*, and this activity was essentially the same against the remaining Gram-negative anaerobes (BMS 284756 MIC₉₀, 2 µg/ml by Etest and AD. Inter-method (Etest versus AD) comparisons showed a high correlation for anaerobes. BMS 284756 activity against Gram-positive anaerobes (61 strains, *C. difficile*) was generally reduced for *C. difficile* (61 strains, MIC₉₀ at >8 µg/ml), but excellent for other species (MIC_{50/90}, 0.5/2 µg/ml). The correlation between test methods was slightly reduced.

Conclusions: BMS 284756 was very active against *C. jejuni*, *H. pylori*, *Legionella* spp. and most anaerobes (*B. fragilis* MIC₉₀, 2 µg/ml), and the Etest proved to be a valuable and accurate alternative method for testing these genera against BMS 284756. The potential role of BMS 284756 for treatment of infections caused by these fastidious species warrant further investigation.

INTRODUCTION

Campylobacter, *Helicobacter* and *Legionella* are fastidious microaerophilic Gram-negative bacilli with a global presence as causes of serious infections. These organisms are implicated in a variety of severe respiratory and gastrointestinal diseases presenting as fever, vomiting, peptic ulcers, abdominal cramping, diarrhea, pneumonia often associated with bacteremia. *Campylobacter jejuni* causes greater than two million gastrointestinal infections per year which is greater than *Salmonella* spp. and *Shigella* spp. combined. Pneumonia with systemic signs is the most frequent form of *Legionella* spp. infection most likely caused by the aerosolization of a contaminated open-water source such as whirlpool spas, humidifiers, misters and showerheads. Severe illness and death rarely occur in Legionellosis among healthy individuals, but more often in patients that are immunosuppressed or those with chronic obstructive pulmonary diseases.

Infections due to anaerobic bacteria are becoming increasingly resistant to older β-lactams due to β-lactamase production and other resistance mechanisms. Metronidazole and clindamycin resistance has been described and isolates described which have been refractory to fluoroquinolone therapy.

BMS 284756 (formerly T-3811), a novel des-fluoro (6) quinolone (Figure 1), has properties of this newer class of quinolones that includes inhibition of many Gram-positive and -negative organisms including anaerobes. Early reports indicate less acute toxicity compared to some previously utilized fluoroquinolones. In this study, the antimicrobial activity of BMS 284756, ciprofloxacin, gatifloxacin, levofloxacin, and trovafloxacin against *C. jejuni*, *H. pylori*, *Legionella* spp., and a sample of anaerobic bacteria were compared using Etest. Also compared were Etest and the reference agar dilution MIC results to determine if the Etest could accurately determine BMS 284756 susceptibility for these pathogens.

MATERIALS AND METHODS

Isolates of *C. jejuni* (38 strains), *H. pylori* (21 strains), *Legionella* spp. (66 strains) and 197 anaerobic species isolates were selected from various collections at the University of Iowa College of Medicine (Iowa City, Iowa) including recent clinical strains from the SENTRY Antimicrobial Surveillance Program (1997-2000). All of these bacteria were stored at -70 °C or below in lysed rabbit blood until processed.

BMS 284756 laboratory standard powder was supplied by Bristol-Myers Squibb (Princeton, NJ) and comparison drugs by their domestic manufacturers. Testing was performed according to the agar dilution method recommended in the NCCLS publication for anaerobic bacteria and modification for other tested species. The initial inoculum of approximately a 1.0 McFarland was applied with a multipoint inoculator (10⁸ CFU/spot) on supplemented Mueller-Hinton or Brucella blood agar plates containing 5% sheep blood for the *Campylobacter* spp., *H. pylori* and anaerobes with a serial two-fold dilution scheme ranging from 0.016 to 8 µg/ml. BCYE agar was supplemented with a defined growth supplement and used for testing the *Legionella* spp. The same suspension was utilized to inoculate the Etestagar plates before the application of the appropriate strips (BMS 284756, ciprofloxacin, levofloxacin, gatifloxacin, and trovafloxacin). Etest strips were obtained from AB BIODISK (Solna, Sweden). The organisms were incubated at 35°C in appropriate microaerophilic or anaerobic atmospheres and MIC values determined at 48 -72 hours.

TABLE 1: Comparative activity of BMS284756 and selected fluoroquinolones tested against recent clinical isolates of *Campylobacter jejuni* (38 strains), *Helicobacter pylori* (21 strains), *Legionella* spp. (66), Gram-negative anaerobes (121) and Gram-positive anaerobes (76) by the Etest method.

Organism (No. Tested)	Antimicrobial Agent	MIC (µg/ml)			% Inhibited at:		
		Range	50%	90%	£ 1	£ 2	£ 4
<i>C. jejuni</i> (38)	BMS284756	0.064->32	0.032	16	89.4	89.4	89.4
	Gatifloxacin	0.016->32	0.032	4.0	89.4	89.4	94.7
	Levofloxacin	0.016->32	0.064	>32	89.4	89.4	89.4
	Trovafloxacin	0.008->32	0.016	8.0	89.4	89.4	89.4
<i>H. pylori</i> (21)	BMS284756	≤0.002-0.032	0.004	0.008	100.0	100.0	100.0
	Gatifloxacin	0.004-0.064	0.016	0.032	100.0	100.0	100.0
	Levofloxacin	0.004-0.125	0.016	0.032	100.0	100.0	100.0
	Trovafloxacin	0.004-0.125	0.016	0.032	100.0	100.0	100.0
<i>Legionella</i> spp. (66)	BMS284756	0.25-20	0.25	0.5	100.0	100.0	100.0
	Ciprofloxacin	0.125-0.25	0.125	0.25	100.0	100.0	100.0
	Gatifloxacin	0.25-1.0	0.25	0.5	100.0	100.0	100.0
	Levofloxacin	0.06-0.5	0.125	0.25	100.0	100.0	100.0
Anaerobes Gram-negative (121)	BMS284756	0.016->32	0.25	2.0	88.4	93.4	94.2
	Gatifloxacin	0.064->32	1.0	16.0	61.2	78.5	85.1
	Levofloxacin	0.064->32	2.0	>32	33.1	62.8	74.4
	Trovafloxacin	0.016->32	0.25	4.0	81.0	86.8	94.9
Gram-positive (76)	BMS284756	0.032->32	0.5	>32	63.2	64.5	68.4
	Gatifloxacin	0.064->32	4.0	>32	21.1	48.7	63.2
	Levofloxacin	0.125->32	>32	>32	13.2	15.8	18.4
	Trovafloxacin	0.032->32	1.0	>32	50.0	64.5	64.5

TABLE 2: Comparison of Etest and agar dilution (AD) test results for BMS 284756 versus strains of *C. jejuni*, Gram-negative anaerobes and Gram-positive anaerobes.

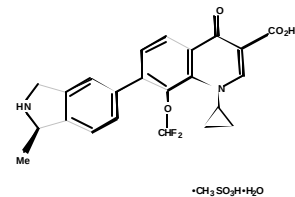
Organism (No. Tested)	Occurrences at Etest / AD MIC ratio:						
	£ 0.125	0.25	0.5	1	2	4	8
<i>C. jejuni</i> (30) %	2 6.7	10 33.3	6 20.0	10 33.3	2 6.7	0 0.0	0 0.0
Gram-negative anaerobes (121) %	2 1.6	43 35.5	49 40.5	18 14.9	6 5.0	2 1.7	1 0.8
Gram-positive anaerobes (76) %	2 2.6	12 15.8	30 39.5	31 40.8	1 1.3	0 0.0	0 0.0

^a Percentage ± two log₂ dilutions was 93.3% (60.0% at ± one log₂ dilution).

^b Percentage ± two log₂ dilutions was 97.6% (60.4% at ± one log₂ dilution).

^c Percentage ± two log₂ dilutions was 97.4% (81.6% at ± one log₂ dilution).

FIGURE 1: Chemical structure of BMS 284756 (formerly T-3811)



RESULTS

- The activity of gatifloxacin and BMS 284756 (MIC₅₀ 0.032 µg/ml) were equal or two-fold greater than levofloxacin (MIC₅₀ 0.064 µg/ml) against the *C. jejuni*, and slightly less active than trovafloxacin (MIC₅₀ 0.016 µg/ml), see Table 1.
- BMS284756 (MIC₉₀ 0.008 µg/ml) was four-fold more active against the *H. pylori* isolates compared to gatifloxacin or trovafloxacin.
- Ciprofloxacin and levofloxacin (MIC₉₀ 0.25 µg/ml) were slightly more potent (two-fold) than either BMS 284756 or gatifloxacin, however all *Legionella* strains were considered susceptible to the quinolones at breakpoints proposed or recommended by the NCCLS.
- Against the Gram-negative anaerobe isolates, BMS 284756 and trovafloxacin (MIC₉₀ 2 and 4 µg/ml, respectively) were four- to eight-fold more active than the other comparison agents (MIC₉₀ ≥ 16 µg/ml).
- The quinolone activity against the Gram-positive anaerobes (80% of strains were *C. difficile*) was generally reduced, but excellent for species (MIC₉₀ 0.5/2 µg/ml) other than *C. difficile*.
- The inter-method (Etest versus AD) comparison of BMS 284756 test results showed a high correlation of 93.3, 97.6 and 97.4% of results within ± two log₂ dilutions for *C. jejuni*, Gram-negative and Gram-positive anaerobes, respectively (Table 2).
- This analysis demonstrated a slight trend towards lower Etest MIC values as compared to the reference agar dilution method, but showed no susceptibility categorical discords when using the proposed BMS 284756 susceptible breakpoint ≤ 4 µg/ml.

CONCLUSIONS

- The BMS 284756 Etest proved to be easy to use and interpret, making this method a practical solution for testing these fastidious isolates in a clinical laboratory setting.
- These results indicate the need for further clinical trials to establish the true effectiveness of des-fluoro (6) quinolones such as BMS 284756 as therapy for infections caused by *C. jejuni*, *H. pylori*, *Legionella* spp., and anaerobic bacteria.

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