

Activity of LBM415 Compared to Those of 11 Other Agents against *Haemophilus* Species

Tatiana Bogdanovich, Kathy A. Smith, Catherine Clark, Glenn A. Pankuch, Gengrong Lin, Pamela McGhee, Bonifacio Dewasse, and Peter C. Appelbaum*

Hershey Medical Center, Hershey, Pennsylvania 17033

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When tested against 254 *Haemophilus influenzae* strains, LBM415, a peptide deformylase inhibitor, gave MIC₅₀ and MIC₉₀ values of 2.0 µg/ml and 8.0 µg/ml, respectively. The MICs were independent of β-lactam or quinolone susceptibility and the presence or absence of macrolide efflux or ribosomal protein mutations. The MICs of LBM415 against 23 *H. parainfluenzae* strains were similar to those against *H. influenzae*. In contrast, erythromycin, azithromycin, and clarithromycin gave unimodal MIC distributions, and apart from β-lactamase-negative, ampicillin-resistant strains, all strains were susceptible to the β-lactams tested. Apart from selected quinolone-resistant strains, all strains were susceptible to ciprofloxacin, levofloxacin, gatifloxacin, moxifloxacin, and gemifloxacin. Resistance to trimethoprim-sulfamethoxazole was common. The potencies of all drugs against 23 *H. parainfluenzae* strains were similar to those against *H. influenzae*. Time-kill studies with 10 *Haemophilus* strains showed LBM415 to be bactericidal at 2× the MIC against 8 of 10 strains after 24 h. For comparison, the macrolides and β-lactams were bactericidal against 8 to 10 strains each at 2× the MIC after 24 h. Quinolones were bactericidal against all 10 strains tested at 2× the MIC after 24 h. Against six *H. influenzae* strains, postantibiotic effects for LBM415 lasted between 0.8 and 2.2 h. In multistep resistance selection studies, LBM415 produced resistant clones in 7 of the 10 strains tested, with MICs ranging from 4 to 64 µg/ml. No mutations in deformylase (*def*) and formyltransferase (*fnt*) genes were detected in any of the LBM415-resistant mutants.

Haemophilus influenzae is an important pathogen responsible for community-acquired respiratory tract infections in children and adults, including pneumonia, acute exacerbations of chronic bronchitis, sinusitis, and otitis media (9, 23). In countries such as the United States, where the *H. influenzae* type b vaccine is widely used, type b has been replaced by untypeable *H. influenzae* strains. *Haemophilus parainfluenzae* strains may also play a role in the pathogenesis of acute exacerbations of chronic bronchitis (S. Sethi, personal communication).

Antimicrobials used for the empirical treatment of *Haemophilus* infections include β-lactams, macrolides, and (in adults) fluoroquinolones. The major resistance mechanism in *H. influenzae* in the United States and Europe is the production of β-lactamase (encoded by *bla*_{TEM-1} and, rarely, *bla*_{ROB-1}), which is present in over 41% of *H. influenzae* strains in the United States (17, 24, 27, 30). Although less common, resistance to β-lactams in the absence of β-lactamase production (β-lactamase-negative ampicillin resistance [BLNAR]) is caused by alterations (mutations) in penicillin-binding protein 3 (8, 31). Strains producing β-lactamases remain susceptible to amoxicillin-clavulanate; however, clavulanate is not active against BLNAR strains. In addition, very rare *H. influenzae* strains producing β-lactamase have been found to be resistant to amoxicillin-clavulanate (8). Among macrolides and azalides, azithromycin yields the lowest MIC against *H. influenzae*, followed by erythromycin and clarithromycin (6, 21, 28). However, the pharmacokinetic and pharmacodynamic properties of

these compounds cast doubt on their clinical efficacy against *H. influenzae* (18), and our group recently reported a macrolide efflux mechanism in baseline strains with MICs in the “susceptible” category (29). At present, most clinical strains of *H. influenzae* are quinolone susceptible (17); however, quinolone resistance has been described for both laboratory and clinical settings (2, 10, 26).

LBM415 is a new peptide deformylase (PDF) inhibitor with excellent activities against streptococci and staphylococci (7, 13, 19, 20). This compound is also active against some gram-negative strains as well as *Mycoplasma pneumoniae* (32) and has some activity against *Legionella pneumophila* (12). This study examines the activities of LBM415 and comparators against *Haemophilus* by microdilution MIC testing, time-kill and postantibiotic effect (PAE) assays, and multistep resistance selection studies.

MATERIALS AND METHODS

Bacteria and MIC testing. For microdilution MIC testing, 254 strains of *H. influenzae* and 23 strains of *H. parainfluenzae* were tested. Strains were isolated from 1997 to 2002 and comprised 6 β-lactamase-positive and 17 β-lactamase-negative *H. parainfluenzae* strains and 102 β-lactamase-positive, 130 β-lactamase-negative, and 22 BLNAR (including 2 β-lactamase-positive, amoxicillin-clavulanate-resistant, 11 quinolone-resistant, 9 macrolide-hypersusceptible, and 14 macrolide-hyperresistant strains) *H. influenzae* strains. Quinolone-resistant strains had previously reported alterations in type II topoisomerase (3, 10). All strains were from clinical specimens, predominantly sputa, bronchial aspirates, blood, and cerebrospinal fluid (the last two were obtained in countries which do not use the *H. influenzae* type b vaccine). Strains were stored at –70°C in double-strength skim milk (Difco Laboratories, Detroit, Mich.) before being tested. β-Lactamase testing was performed by the nitrocefin disk method (Ceftinase; BBL Microbiology Systems, Inc., Cockeysville, Md.).

MIC testing was performed by the CLSI (former NCCLS) microdilution method (25), using freshly prepared *Haemophilus* test medium (HTM) in com-

* Corresponding author. Mailing address: Department of Pathology, Hershey Medical Center, Hershey, PA 17033. Phone: (717) 531-5113. Fax: (717) 531-7953. E-mail: pappelbaum@psu.edu.

mercially prepared frozen trays (TREK, Inc., Cleveland, OH) (1, 14). LBM415 was obtained from Novartis Laboratories, Hanover, NJ. Other compounds were obtained from their respective manufacturers. Inocula were prepared from chocolate agar plates incubated for 20 to 24 h by the direct colony suspension method. The final well concentration was approximately 5×10^5 CFU/ml. The standard quality control strains *H. influenzae* ATCC 49766 and *H. influenzae* ATCC 49247 were used in each run. Inoculum checks were done, and only suspensions yielding 3×10^5 to 7×10^5 CFU/ml were used. Trays were covered and incubated at 35°C in air. No susceptibility breakpoints for LBM415 currently exist.

Time-kill studies. For time-kill testing, nine *H. influenzae* (three β -lactamase-positive, three β -lactamase-negative [including one macrolide-hypersusceptible strain], and three BLNAR strains) and one β -lactamase-positive *H. parainfluenzae* strain were tested. Time-kill studies were done as described previously (28). Briefly, glass tubes containing 5 ml of freshly made HTM with doubling antibiotic concentrations were inoculated with approximately 5×10^5 to 5×10^6 CFU/ml and incubated at 35°C in a shaking water bath. Viability counts of antibiotic-containing suspensions were performed at 0, 3, 6, 12, and 24 h by plating 10-fold dilutions (in HTM) of 0.1-ml aliquots from each tube on chocolate agar plates. Recovery plates were incubated for up to 48 h. Colony counts were performed on plates yielding 30 to 300 colonies. The lower limit of sensitivity of colony counts was 300 CFU/ml. The results were analyzed by determining the numbers of strains which yielded $\Delta(\log_{10}$ CFU/ml) values of -1 (corresponding to 90% killing), -2 (99% killing), and -3 (99.9% killing) at 3, 6, 12, and 24 h compared to counts at 0 h. A given concentration of antibacterial (expressed as a multiplicity of the MIC) was considered bactericidal if it reduced the inoculum size $\geq 3 \log_{10}$ CFU/ml and bacteriostatic if the inoculum size was reduced $< 3 \log_{10}$ CFU/ml during a specific time period. With the sensitivity threshold and inocula employed in this study, no problems were encountered in delineating 99.9% killing. Issues of drug carryover were addressed by dilution, as we described previously (28).

PAE studies. PAE studies were done with three β -lactamase-positive, two β -lactamase-negative, and one BLNAR *H. influenzae* strain. PAE was determined by the viable plate count method, using freshly prepared HTM. The PAE was induced by exposure to $10\times$ the MICs of all compounds for 1 h. For PAE testing, tubes containing 5 ml broth with antibiotic were inoculated with approximately 5×10^6 CFU/ml. Inocula were prepared by suspending growth from an overnight chocolate agar plate in broth. Growth controls with inocula but no antibiotic were included with each experiment. Inoculated test tubes were placed in a shaking water bath at 35°C for an exposure period of 1 h. At the end of the exposure period, cultures were diluted 1:1,000 in prewarmed broth to remove the antibiotic by dilution. Antibiotic removal was confirmed by comparing the growth curve of a control culture containing no antibiotic to another at $0.001\times$ the exposure concentration. Viability counts were determined before exposure, immediately after dilution (0 h), and then every 2 h until the turbidity of the tube reached a no. 1 McFarland standard. The PAE was defined as follows: $PAE = T - C$, where T is the time required for viability counts of an antibiotic-exposed culture to increase $1 \log_{10}$ above counts immediately after dilution and C is the corresponding time for growth of the control (5).

Multistep selection studies. For resistance selection studies, four β -lactamase-positive and six β -lactamase-negative strains of *H. influenzae* (including one BLNAR strain with penicillin-binding protein 3 changes and one macrolide-hypersusceptible strain lacking efflux with an azithromycin MIC of 0.25 μ g/ml) were tested. The method was a modification of that described previously by our group (3). Briefly, serial passages in freshly prepared HTM were performed daily for each strain with subinhibitory concentrations of all antimicrobials, with a starting inoculum of approximately 1×10^8 CFU/ml. For each subsequent daily passage, an inoculum was taken from the tube nearest the MIC, usually one to two dilutions below, which had the same turbidity as the antibiotic-free controls. This inoculum was used to determine the next MIC. A minimum of 14 passages were performed in each case, unless a >4 -fold increase in the MIC was seen, in which case subculturing in the presence of antibiotic ceased. It is important to realize that the latter criterion, in view of the lack of susceptibility breakpoints for LBM415, was arbitrarily based upon our previous studies (3, 21). The maximal number of passages was 50. The stability of the acquired resistance was determined after 10 daily passages of the clone on chocolate agar (BBL) without antibiotics. The identities of parental and mutant clones were confirmed by pulsed-field gel electrophoresis (using SmaI) in a CHEF DR III apparatus (Bio-Rad, Hercules, CA). The MIC of each drug for each resistant clone was determined by standard macrodilution methodology. Resistance mechanisms were determined for resistant clones and parent strains as described below.

Mechanism of resistance. Since mutations in the deformylase (*def*) and formyltransferase (*fnt*) genes were shown to be responsible for resistance to deformylase inhibitors in *Streptococcus pneumoniae* (22) and *Staphylococcus*

TABLE 1. MICs (μ g/ml) of compounds against 254 *H. influenzae* and 23 *H. parainfluenzae* strains

Antibiotic	β -Lactamase positive (<i>n</i> = 102)			β -Lactamase negative (<i>n</i> = 130)			BLNAR (<i>n</i> = 22)			All strains (<i>n</i> = 254)			MIC for <i>H. parainfluenzae</i> (<i>n</i> = 23)		
	Range	MIC ₅₀ /MIC ₉₀	MIC ₅₀ /MIC ₉₀	Range	MIC ₅₀ /MIC ₉₀	MIC ₅₀ /MIC ₉₀	Range	MIC ₅₀ /MIC ₉₀	MIC ₅₀ /MIC ₉₀	Range	MIC ₅₀ /MIC ₉₀	MIC ₅₀ /MIC ₉₀	Range	MIC ₅₀ /MIC ₉₀	MIC ₅₀ /MIC ₉₀
LBM415	0.06–16	2/8	2/4	0.03–>16	1/8	1/8	0.12–>16	1/8	1/8	0.03–>16	2/8	2/4	0.25–8	2/4	2/4
Erythromycin	0.5–>64	4/8	4/16	0.06–>64	4/16	4/16	2–16	4/16	4/16	0.06–>64	4/16	4/8	1–8	4/8	4/8
Azithromycin	0.12–>64	1/2	1/2	≤ 0.03 –>64	1/2	1/2	0.25–4	1/2	1/2	≤ 0.03 –>64	1/2	0.5/1	0.06–2	0.5/1	0.5/1
Clarithromycin	1–>64	8/16	8/16	≤ 0.03 –>64	8/16	8/16	2–32	4/16	4/16	≤ 0.03 –>64	8/16	8/16	0.5–16	4/16	4/16
Amoxicillin	8–>32	>32/>32	5/2	0.12–8	5/2	8/16	1–>32	8/16	8/16	0.12–>32	4/16	4/16	0.06–>32	0.5/>32	0.5/>32
Amoxicillin-clavulanate	0.12–8	1/4	0.5/2	0.12–8	0.5/2	0.5/2	1–>32	8/16	8/16	0.12–>32	0.5/4	0.5/4	0.06–>32	0.5/4	0.5/4
Cefuroxime	0.015–4	0.06/0.25	0.06/0.25	0.03–2	0.06/0.25	0.06/0.25	0.25–>16	2/4	2/4	0.015–>16	0.06–0.5	0.03/0.5	≤ 0.008 –>16	0.03/0.5	0.03/0.5
Cefuroxime	0.25–32	1/2	1/2	0.12–16	1/2	1/2	1–>64	4/64	4/64	0.12–>64	1/4	0.25/2	0.12–>64	0.25/2	0.25/2
Cefdinir	0.12–2	0.25/1	0.25/1	0.06–4	0.25/1	0.25/1	0.5–>16	2/16	2/16	0.06–>16	0.5/1	0.12/1	0.06–>16	0.12/1	0.12/1
Ciprofloxacin	0.008–>2	0.008/0.015	0.008/0.015	0.004–>2	0.008/0.015	0.008/0.015	0.004–>2	0.008/0.015	0.008/0.015	0.004–>2	0.008/0.015	0.008/0.015	0.004–0.03	0.004–0.03	0.008/0.015
Levofloxacin	0.008–2	0.015/0.015	0.015/0.015	0.004–4	0.015/0.015	0.015/0.015	0.008–2	0.015/0.015	0.015/0.015	0.004–4	0.015/0.015	0.015/0.015	0.004–0.06	0.004–0.06	0.015/0.015
Gatifloxacin	0.004–8	0.008/0.015	0.008/0.015	0.002–2	0.008/0.015	0.008/0.015	0.004–1	0.008/0.015	0.008/0.015	0.002–2	0.002/0.008	0.002/0.008	0.004–0.12	0.004–0.12	0.015/0.015
Moxifloxacin	0.008–>2	0.015/0.015	0.015/0.015	0.008–2	0.015/0.015	0.015/0.015	0.008–2	0.015/0.015	0.015/0.015	0.008–2	0.015/0.015	0.015/0.015	0.015–0.25	0.015–0.25	0.06/0.12
Gemifloxacin	≤ 0.001 –2	0.002/0.008	0.002/0.008	≤ 0.001 –2	0.002/0.008	0.002/0.008	≤ 0.001 –0.5	0.002/0.008	0.002/0.008	≤ 0.001 –2	0.002/0.008	0.002/0.008	0.004–0.06	0.004–0.06	0.008/0.015
Trimethoprim-sulfamethoxazole	≤ 0.03 –>16	0.5/16	0.5/16	0.12–>16	0.5/16	0.5/16	≤ 0.03 –>16	0.5/16	0.5/16	≤ 0.03 –>16	0.5/16	0.5/16	0.06–>16	0.06–>16	0.5/16
Tetracycline	0.12–32	0.25/0.5	0.25/0.5	≤ 0.06 –1	0.25/0.5	0.25/0.5	0.12–0.5	0.25/0.5	0.25/0.5	≤ 0.06 –32	0.25/0.5	0.25/0.5	0.25–16	0.25–16	0.5/1

TABLE 2. Time-kill analyses of 10 *Haemophilus* strains^a

Drug and dose (multiplicity of MIC)	No. of strains killed at indicated time (h) ^b											
	3			6			12			24		
	-1	-2	-3	-1	-2	-3	-1	-2	-3	-1	-2	-3
LBM415 (MICs, 0.125–4 µg/ml)												
4	2	1	0	7	3	1	10	8	6	10	10	9
2	1	1	0	5	3	0	9	5	5	10	10	8
1	1	0	0	4	0	0	6	4	3	10	8	5
Erythromycin (MICs, 0.5–8 µg/ml)												
4	7	3	0	9	6	3	10	10	8	10	10	10
2	6	0	0	8	5	2	10	9	5	10	10	9
1	3	0	0	6	2	0	9	6	4	10	8	7
Azithromycin (MICs, 0.125–2 µg/ml)												
4	7	4	1	9	7	3	10	10	10	10	10	10
2	7	3	1	7	6	3	10	9	8	10	10	9
1	4	1	0	5	3	2	8	7	3	10	9	6
Clarithromycin (MICs, 0.25–8 µg/ml)												
7	7	2	0	8	6	2	10	10	6	10	10	10
4	3	2	0	6	4	1	9	8	4	10	10	10
2	2	0	0	5	3	1	7	4	3	9	6	4
1												
Amoxicillin-clavulanate (MICs, 0.25–8 µg/ml)												
4	4	0	0	7	1	0	10	9	2	10	10	10
2	2	0	0	7	1	0	10	8	2	10	10	10
1	2	0	0	5	1	0	9	6	2	10	9	8
Cefpodoxime (MICs, 0.06–2 µg/ml)												
4	3	0	0	9	2	0	10	10	2	10	10	10
2	1	0	0	6	2	0	10	8	2	10	10	10
1	0	0	0	6	1	0	9	6	1	10	9	6
Cefuroxime (MICs, 0.5–16 µg/ml)												
4	3	0	0	9	1	0	10	9	4	10	10	10
2	2	0	0	8	1	0	10	8	3	10	10	10
1	2	0	0	5	1	0	9	6	2	10	9	8
Cefdinir (MICs, 0.25–4 µg/ml)												
4	1	0	0	10	2	0	10	9	2	10	9	9
2	2	0	0	8	1	0	10	8	2	10	9	8
1	1	0	0	6	1	0	10	5	0	9	7	4
Ciprofloxacin (MICs, 0.008–0.015 µg/ml)												
4	9	4	0	10	6	2	10	10	7	10	10	10
2	8	2	0	10	4	1	10	10	6	10	10	10
1	3	1	0	9	1	1	1	8	3	10	10	10
Levofloxacin (MICs, 0.008–0.03 µg/ml)												
4	10	5	1	10	8	2	10	10	9	10	10	10
2	9	3	0	10	7	1	10	10	8	10	10	10
1	8	0	0	9	4	0	9	9	4	9	9	9
Gatifloxacin (MICs, 0.004–0.03 µg/ml)												
4	10	7	0	10	10	4	10	10	9	10	10	10
2	9	3	0	10	6	2	10	10	7	10	10	10
1	4	1	0	8	2	1	10	9	2	9	9	8
Moxifloxacin (MICs, 0.008–0.06 µg/ml)												
4	10	5	3	10	9	6	10	9	9	10	10	10
2	9	4	1	10	8	2	10	8	8	10	10	10
1	4	2	0	6	3	1	10	7	3	10	10	9

^a Nine *H. influenzae* and one *H. parainfluenzae* strain.

^b -1, 90% killing; -2, 99% killing; -3, 99.9% killing.

aureus (4), both the *def* and *fmt* genes were amplified from LBM415-resistant mutants by using the following primers: DefHinF, 5'-TCCTGAGATCACTTA AAGGTTG-3'; DefHinR, 5'-AGCTTTTCGCAATCTGCTTC-3'; FmtHinF, 5'-TTTGCTGCACAGCATTTACA-3'; and FmtHinR, 5'-TTCTGCACGGCC ATTTAATA-3'. The presence of mutations in the L4 and L22 protein genes and in 23S rRNA (for macrolide- and telithromycin-resistant clones) and of quinolone resistance determinants (for moxifloxacin-resistant clones) was examined by using previously described primers and conditions (3, 29). PCR products were purified using a QIAquick PCR purification kit (QIAGEN, Valencia, Calif.) and sequenced using a CEQ8000 genetic analysis system (Beckman Coulter, Fullerton, CA).

RESULTS

When tested against 254 *H. influenzae* strains, LBM415, a peptide deformylase inhibitor, gave MIC₅₀ and MIC₉₀ values of 2.0 µg/ml and 8.0 µg/ml, respectively (Table 1). The MICs were independent of β-lactam or quinolone susceptibility and the presence or absence of macrolide efflux or ribosomal protein mutations. The MICs of LBM415 against 23 *H. parainfluenzae* strains were similar to those against *H. influenzae* (Table 1). Of nine strains with LBM415 MICs of ≥16.0 µg/ml, two strains were BLNAR, including one quinolone-resistant strain, three strains were β-lactamase positive, and four strains were β-lactamase negative. Macrolides gave the usual unimodal distributions, and quinolone MICs of resistant strains with known mutations in quinolone resistance-determining regions (10) ranged between 0.125 and >2.0 µg/ml. Cefpodoxime was the most potent cephalosporin tested, and gatifloxacin and gemifloxacin were the most potent quinolones. Resistance to both trimethoprim-sulfamethoxazole and tetracycline sometimes occurred (Table 1).

Time-kill analyses are presented in Table 2. LBM415 was bactericidal (99.9% killing) against 8 of 10 *Haemophilus* strains tested after 24 h of incubation at 2× the MIC. Erythromycin and azithromycin were bactericidal against 9 strains, and clarithromycin was bactericidal against 10 strains after 24 h of incubation at 2× the MIC. All four quinolones, amoxicillin-clavulanate, cefpodoxime, and cefuroxime were bactericidal against all strains at 2× the MIC after 24 h. No differences for LBM415 were observed in the bactericidal activities of antibacterials tested based on β-lactamase production or BLNAR phenotypes. The time-kill activity for the single *H. parainfluenzae* strain tested was similar to those for the nine *H. influenzae* strains (data not shown).

The PAE results for six *H. influenzae* strains are presented in Table 3. As shown, the times of PAE for LBM415 ranged between 0.8 and 2.2 h. β-Lactam times of PAE ranged between 0 and 1.8 h, while those for quinolones (1.6 to 6.2 h) and macrolides (2.0 to 9.2 h) were longer.

The results of multistep resistance selection are given in Table 4. LBM415 yielded resistant clones after 14 to 20 days in 7/10 strains, with MICs rising from 0.5 to 16 µg/ml (parents) to 4 to 64 µg/ml (resistant clones). Azithromycin had resistant clones (from 0.25 to 2 µg/ml to 4 to >64 µg/ml) in 8/9 strains tested in 15 to 47 days. Moxifloxacin had resistant clones, as defined above (from 0.016 to 0.125 µg/ml to 0.06 to 0.5 µg/ml), in 5/10 strains in 26 to 39 days. Amoxicillin-clavulanate and cefpodoxime yielded no resistant clones in any of the strains tested at the maximum of 50 passages.

Selected LBM415-, azithromycin-, clarithromycin-, erythromycin-, and moxifloxacin-resistant clones were checked for

TABLE 3. MICs and PAEs of six *H. influenzae* strains

Drug	MIC range (µg/ml)	Mean PAE (range) (h) ^a
LBM415	0.5–4.0	1.2 (0.8–2.2)
Erythromycin	2.0–8.0	3.1 (2.0–4.4)
Azithromycin	0.5–2.0	7.1 (5.6–9.2)
Clarithromycin	2.0–8.0	3.4 (2.4–4.6)
Amoxicillin-clavulanate	0.25–1.0	0.3 (0.0–0.9)
Cefpodoxime	0.06–0.25	0.2 (0.1–0.6)
Cefuroxime	0.5–2.0	0.6 (0.1–1.4)
Cefdinir	0.25–0.5	0.6 (0.2–1.8)
Ciprofloxacin	0.004–0.015	2.2 (1.6–3.4)
Levofloxacin	0.008–0.015	3.8 (2.8–5.0)
Gatifloxacin	0.004–0.015	3.4 (1.9–6.2)
Moxifloxacin	0.008–0.03	3.6 (2.4–5.4)

^a PAE induced by 1 h of exposure at 10× the MIC.

cross-resistance to other compounds (Table 4). All (8/8) azithromycin-resistant mutants had 8 to 32× increases in the MIC toward erythromycin, and seven of them had these increases in the MIC toward clarithromycin. Four of nine erythromycin-selected mutants had increased MICs (8×) towards clarithromycin; all (7/7) clarithromycin-resistant mutants demonstrated 8 to 16× increases in erythromycin MICs. In addition to the above, several instances of increases in the MIC of >2 log₂ dilution steps for structurally unrelated antibiotics were noted. Thus, five of seven LBM415 mutants had 8 to 32× increases in the MIC toward erythromycin, while one clarithromycin-resistant clone and one moxifloxacin-resistant clone had an 8× increase in LBM415 MIC compared to the corresponding parent strains. The majority of mutants had stable resistance phenotypes after repeated subculturing in the absence of antibiotic. The two exceptions were an LBM415-resistant clone derived from strain 153-026 (MIC dropped from 64 µg/ml to 16 µg/ml) and an azithromycin-resistant mutant derived from strain 153-008 (MIC decreased from >64 µg/ml to 32 µg/ml). However, in both of these cases, the LBM415 MICs for resistant clones remained high.

No mutations were detected in either the *def* or *fmt* genes of LBM415-resistant clones. Various mutations were found in the L4 and L22 ribosomal proteins of seven macrolide-resistant clones. No mutations in 23S rRNA were detected in any of the macrolide-resistant mutants. Moxifloxacin-resistant mutants had mutations in GyrA, ParC, and GyrB.

DISCUSSION

LBM415 is a novel member of an existing class of antimicrobials inhibiting bacterial PDF, which deformylates the *N*-formylmethionine of newly synthesized polypeptides. It has preliminarily been considered for treatment of community-acquired respiratory tract infections due to its proven activity against gram-positive cocci (pneumococci and staphylococci) as well as the atypical pathogens *M. pneumoniae* and *L. pneumophila* (7, 12, 13, 32). In the current study, LBM415 gave MICs between 0.03 and 8.0 µg/ml for 245 of 254 *H. influenzae* and all 23 *H. parainfluenzae* strains tested. Of the nine isolates with LBM415 MICs of >16.0 µg/ml, four were β-lactamase negative, three were β-lactamase positive, and two were BLNAR. Overall, our results are similar to those obtained

TABLE 4. Results of multistep resistance selection by LBM415 and comparators^a

Strain	Drug ^b	MIC (µg/ml)		Day of resistance	Cross-resistance MIC (µg/ml)						
		Initial	Resistance		LBM	AZM	ERY	CLR	AMX	CPD	MOX
110-019	LBM	1	8	17	4	2	32	16	1	0.25	0.125
	AZM	1	8	15	1	8	>32	16	2	0.25	0.06
	ERY	4	32	14	4	2	16	32	2	0.25	0.125
	CLR	4	32	21	4	2	64	32	2	0.25	0.125
	MOX	0.06	0.5	39	1	1	16	8	2	0.25	0.25
112-048	LBM	4	16	50							
	AZM	1	8	34	16	8	>32	32	1	0.25	0.03
	ERY	4	64	21	16	2	32	32	2	0.25	0.03
	CLR	4	32	24	4	2	64	16	1	0.06	0.03
	MOX	0.016	0.125	32	16	2	>64	16	1	0.125	0.125
621-049	LBM	2	16	20	8	2	>32	16	0.5	0.125	0.125
	AZM	2	16	47	4	16	>64	64	0.5	0.125	0.125
	ERY	8	>64	31	4	4	>64	32	0.5	0.25	0.06
	CLR	8	16	50							
	MOX	0.06	0.5	26	4	2	32	8	0.5	0.125	0.5
HI 30	LBM	0.5	4	15	2	0.25	32	2	1	0.125	0.06
	AZM	0.25	4	20	2	4	32	8	2	0.25	0.06
	ERY	1	32	14	0.25	0.5	32	8	4	0.25	0.03
	CLR	1	8	14	1	0.5	16	8	4	0.5	0.03
	MOXI	0.03	0.25	35	4	1	16	8	4	0.25	0.25
153-008	LBM	1	8	14	8	2	32	8	0.5	0.06	0.03
	AZM	1	>64	34	2	32	>32	>64	0.5	0.125	0.03
	ERY	4	64	21	2	2	32	8	0.5	0.06	0.03
	CLR	4	32	46	2	2	32	16	0.5	0.125	0.03
	MOX	0.03	0.06	50							
110-061	LBM	1	8	15	8	2	>32	16	1	0.25	0.06
	AZM	1	16	22	2	16	>32	>64	1	0.25	0.06
	ERY	8	64	25	4	2	64	64	2	0.25	0.06
	CLR	8	64	40	8	2	64	64	1	0.5	0.06
	MOX	0.03	0.25	34	2	1	16	8	1	0.25	0.5
204-001	LBM	8	8	50							
	AZM	1	1	50							
	ERY	4	32	31	16	1	16	8	0.25	0.04	0.25
	CLR	4	8	50							
	MOX	0.125	0.25	50							
113-012	LBM	16	32	50							
	AZM	2	4	50							
	ERY	16	>64	31	16	4	>64	32	2	0.25	0.03
	CLR	16	32	50							
	MOX	0.03	0.06	50							
153-026	LBM	4	64	15	16	2	32	8	0.5	0.06	0.03
	AZM	2	32	24	2	64	>32	>64	0.5	0.125	0.03
	ERY	8	64	14	8	2	32	32	0.5	0.125	0.03
	CLR	8	64	48	4	4	64	32	0.25	0.06	0.03
	MOX	0.03	0.06	50							
204-018	LBM	4	32	20	32	2	>32	16	0.5	0.125	0.03
	AZM	2	64	17	4	64	>64	>32	0.5	0.125	0.03
	ERY	16	>64	31	8	4	64	64	0.5	0.125	0.03
	CLR	8	64	31	4	4	>64	64	1	0.25	0.03
	MOX	0.03	0.06	50							

^a Amoxicillin-clavulanate and cefpodoxime yielded no resistant clones in any of the strains tested at the maximum of 50 passages. Their cross-resistance patterns to clones selected by other agents are provided for completeness.

^b LBM, LBM415; AZM, azithromycin; ERY, erythromycin; CLR, clarithromycin; MOX, moxifloxacin.

recently by Fritsche and coworkers, who reported an MIC range for LBM415 between 0.03 and 16 µg/ml against 170 ampicillin-susceptible *H. influenzae* strains, with an MIC₅₀ of 1 µg/ml and an MIC₉₀ of 4 µg/ml (15). When LBM415 was tested against 130 ampicillin-resistant strains, the MIC range was 0.5 to 32 µg/ml, with an MIC₅₀ of 2 µg/ml and an MIC₉₀ of 8 µg/ml. The results for other compounds were the same as those found in other studies (6, 10, 16, 21).

Time-kill analyses showed LBM415 to be more bactericidal

against *H. influenzae* than has been reported for pneumococci and staphylococci (7, 13, 15). In general, kill kinetics were similar to those of the β-lactams, macrolides, and quinolones tested. LBM415, similar to tested cephalosporins, had a shorter duration of PAE than did quinolones, especially macrolides and azalide.

Prolonged repeated exposure of *H. influenzae* to LBM415 led to the development of resistance in 7 of the 10 *Haemophilus* strains tested. LBM415 MICs for the obtained clones

ranged from 4 to 64 $\mu\text{g/ml}$. Although it was shown previously that resistance to peptide deformylase inhibitors in *Escherichia coli* or *Staphylococcus aureus* is due to inactivation of transformylase (*fnt* gene) activity (4) and that in *S. pneumoniae* is due to mutations in a deformylase (*defB*) gene (22), no mutations in either deformylase or formyltransferase genes were detected in any LBM415-resistant mutants. However, a recent report by Dean et al. suggests that the AcrAB-TolC efflux pump may play an important role in determining resistance to LBM415 in *H. influenzae* (11). They showed that insertional inactivation of the *acrB* or *tolC* gene in both laboratory and several clinical *H. influenzae* isolates with atypically reduced susceptibilities to LBM415 (MICs of $\geq 16 \mu\text{g/ml}$) significantly increased bacterial susceptibility to LBM415. They also found various mutations in the *acrR* genes, encoding the putative repressor of pump gene expression, from several of these strains and demonstrated increased expression of pump genes in *acrR* mutants selected by exposure to LBM415 at 8 $\mu\text{g/ml}$. The presence of an efflux pump in the parents and clones selected by LBM415 in the current study is under investigation.

In conclusion, it is important to note that LBM415 is no longer being developed and that no pharmacokinetic/pharmacodynamic data are available to establish valid breakpoints for this compound. It is possible that had these been available, MICs for LBM415 against *Haemophilus* might have been too high to permit clinical application. However, it is likely that other new PDF inhibitors with MICs against *H. influenzae* lower than those of LBM415 will follow. Since the mechanisms of action of all PDF inhibitors are similar, the results of this study may act as a representative base from which to work and may indicate a potential role for newer PDF inhibitors in the treatment of community-acquired respiratory tract infections caused by *Haemophilus* spp.

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