Comparisons of Intermethod Susceptibility Testing Accuracy for LBM415 (NVP PDF-713) Using 2,625 Recent Clinical Isolates

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TR FRITSCHE, PR RHOMBERG, RN JONES
The JONES Group/JMI Laboratories, North Liberty, IA

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

ABSTRACT

Background: LBM415 (415), also known as NVP PDF-713, is among the first peptide deformylase inhibitors to enter clinical trials for treatment of community-acquired respiratory tract and cutaneous infections. As reliable MIC and disk testing methods and interpretive criteria are needed to support clinical development, inter-method comparison studies of reference tests were undertaken.
Methods: A NCCLS M23-A2 study using the error rate-bounded (ERB) method was undertaken. Disk diffusion and MIC testing were performed according to NCCLS M2-A8 and M7-A6 reference methods (2003). The collection of 2,625 strains consisted of staphylococci (537 strains); enterococci (515); *S. pneumoniae* (504); other streptococci (500); *H. influenzae* (501); other species (68). Previous studies indicated that a 30-µg 415 disk produced reproducible zone diameters for all targeted species.
Results: Using targeted species, susceptible (S) breakpoints were selected based on available PK/PD results, inter-method regression and ERB analysis. S and resistant (R) MIC breakpoints of ≤ 4 and ≥ 16 µg/ml with correlate zones of ≥ 20 mm and ≤ 16 mm would clearly and accurately divide the potentially treatable (S) and 415-R populations and minimize errors: very major error (false-S) = 1/2,625 or 0.04%; major error (false-R) = 0/2,625 or 0.00%; and minor error = 81/2,625 or 3.09%. *H. influenzae* strains that cluster near the proposed breakpoint produced most errors, as did some non-fermentative Gram-negative bacilli. The overall error rate was only 3.13%, well within acceptable limits as specified by M23-A2.
Conclusions: For staphylococci, streptococci, *S. pneumoniae* and enterococci, no isolate was identified with MIC or zone diameter results that would indicate R to LBM415. For the first three groups, only a S interpretive category might be considered until R strains are detected. However, enterococci and *H. influenzae* should have S, intermediate and R criteria dictated by PK/PD and MIC population considerations.

INTRODUCTION

The peptide deformylase inhibitors have become an attractive new class of antimicrobial agents targeting a previously unutilized mechanism of action. LBM415 (NVP PDF-713; Figure 1) has been extensively studied in vitro and demonstrated potent activity against *Staphylococcus aureus* (oxacillin-susceptible and -resistant strains), coagulase-negative staphylococci (CoNS; oxacillin-susceptible and -resistant strains), enterococci including glycopeptide-resistant strains, *Streptococcus pneumoniae* (penicillin- or macrolide-resistant strains), other streptococcal spp., *Haemophilus* spp., *Moraxella catarrhalis* and some uncommonly isolated species of non-fermentative Gram-negative bacilli.

To establish test in vitro susceptibility accuracy and tentative interpretive criteria for MIC and disk diffusion susceptibility testing methods in support of LBM415 clinical trials, inter-method comparison studies of reference broth microdilution results and zone diameter measurements were undertaken following the National Committee for Clinical Laboratory Standards (NCCLS) M23-A2 guideline [2001]. These studies used 2,625 recent facultative clinical isolates, including 2,557 organisms considered to be within the spectrum of activity for LBM415.

MATERIALS AND METHODS

Specimen Collection. The collection (2,625 strains total) consisted of staphylococci (537 strains, including many oxacillin-resistant *S. aureus* and CoNS); enterococci (515 strains, including 34 vancomycin-resistant *Enterococcus faecalis* and 61 vancomycin-resistant *E. faecium* among 98 vancomycin-non-susceptible isolates), *S. pneumoniae* (504 strains, including many penicillin-resistant isolates), ß-haemolytic streptococci (255 strains), viridans group streptococci (245 strains, many were penicillin-non-susceptible), *H. influenzae* (501 strains), Enterobacteriaceae (28 strains), *Pseudomonas aeruginosa* (10 strains), *Burkholderia cepacia* complex (2 strains), *Stenotrophomonas maltophilia* (4 strains), *Acinetobacter* spp. (6 strains), *Moraxella catarrhalis* (2 strains), two strains each of *P. multocida*, *Micrococcus* spp., *Listeria* spp., *H. parainfluenzae, Gemella* spp., *Bacillus* spp. and *Aerococcus* spp. (14 total isolates), and one strain each of *Leuconostoc* spp. and *Lactobacillus* spp.

Susceptibility Testing. The study was designed using NCCLS M23-A2 guidelines, with analysis performed by the error rate-bounded (ERB) method. Susceptibility test methods included the NCCLS M2-A8 disk diffusion method using 30-µg LBM415 disks prepared by Oxoid (labelled PDI-30) and the NCCLS M7-A6 broth microdilution method using panels produced by TREK Diagnostics (Cleveland, OH). Preliminary susceptibility testing studies indicated that the 30-µg disk for LBM415 produced large, reproducible zone diameters for all targeted species. Quality control guidelines for both MIC and disk diffusion testing for LBM415 have been recently published using the NCCLS M23-A2 study design; quality control strains utilized included *Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 35218, *S. aureus* ATCC 29213, *S. pneumoniae* ATCC 49619, *E. faecalis* ATCC 29212 and *H. influenzae* ATCC 49247; all control results were within recommended limits.

RESULTS

- LBM415 displayed excellent activity against all staphylococci (MIC_{90}, 2 µg/ml), streptococci (MIC_{90}, 1 µg/ml) and enterococci (MIC_{90}, 4 µg/ml) (Table 1). Only *H. influenzae* strains had elevated MIC values at or near the proposed susceptible breakpoints of ≤ 4 or ≤ 8 µg/ml.
- A scattergram comparing all LBM415 MIC values and zone diameters around the commercial 30-µg disks is shown in Figure 2. Of all strains tested only 68 (other organisms, Table 1) were initially considered to be outside the spectrum of LBM415 or did not have specific interpretive criteria in NCCLS documents. Some of these strains, however, had MIC values at ≤ 8 µg/ml.
- Tentative susceptibility breakpoints were based on MIC population distributions and correlate zone diameters along with preliminary PK/PD results [W. Craig, personal communications]. Figure 2 illustrates that selected susceptible and resistant breakpoints of ≤ 4 and ≥ 16 µg/ml with correlate zones of ≥ 20 mm and ≤ 16 mm would clearly and accurately divide the potentially LBM415 treatable and resistant organism populations.
- Using such interpretive criteria, the overall disk diffusion error rates were: very major error (false-susceptible), 0.04%; major error (false-resistant), 0.00%; and minor error, 3.09% for an overall error rate of only 3.13%, well within the acceptable limits specified by NCCLS M23-A2.
- A scattergram comparing LBM415 MIC results to disk diffusion test zone diameters for staphylococci is shown in Figure 3. The range of LBM415 MIC values was ≤ 0.016 to 4 µg/ml and the smallest zone diameter was 24 mm. No intermethod errors were detected using the proposed breakpoint; all organisms were considered susceptible.
- All LBM415 MIC values for *S. pneumoniae* were ≤ 2 µg/ml (zones at ≥ 21 mm; Figure 4), and were ≤ 4 µg/ml (zones at ≥ 21 mm; Figure 5) for all other ß-haemolytic or viridans group streptococci. No interpretive errors were observed when using the tentative breakpoints.
- Enterococci tested by MIC (range 0.06 to 8 µg/ml; MIC_{90}, 4 µg/ml) and disk diffusion methods showed only minor errors (5.05%; Figure 6).
- LBM415 MIC results for *H. influenzae* varied widely from 0.06 to 32 µg/ml and displayed some linear correlation (r = 0.80) between MIC values and inhibitory zones (Figure 7). Calculated error rates for *H. influenzae* were: very major and major errors, 0.00%; and minor errors, 10.38%. All error rates were minor and within acceptable limits (NCCLS).
- A scattergram comparing LBM415 MIC results with zones of inhibition when tested against 68 strains of bacteria from other species/genera (listed in Table 1) is shown in Figure 8. Some organisms were very resistant whereas numerous other uncommonly isolated non-fermentors and unusual Gram-positive isolates had lower MIC results (0.12 - 16 µg/ml). Also *M. catarrhalis* isolates were considered susceptible to LBM415. The correlation coefficient was very high (r = 0.97) and only three minor errors (4.41%) and one very major error (1.47%) were observed.

FIGURE 1. Chemical structure of LBM415.

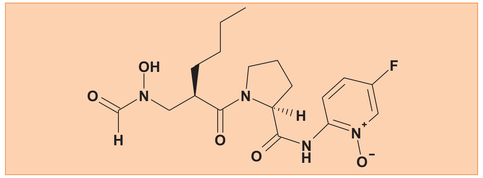
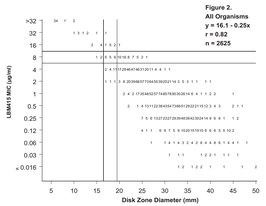
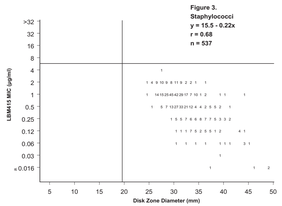


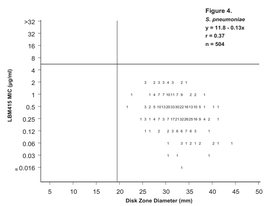
TABLE 1. Antimicrobial activity of LBM415 tested against a collection of 2,625 recent clinical isolates used to develop interpretive criteria for the NCCLS M2-A8 and M7-A6 methods.

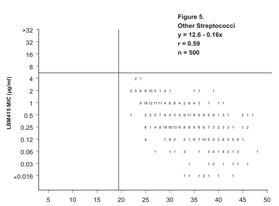
Organism (no. tested)	MIC (µg/ml) 50%	90%	Range	Cumulative % inhibited at (MIC in µg/ml): ≤1	2	4	8
Staphylococci (537)	1	2	≤0.016-4	87.3	99.8	100.0	-
S. pneumoniae (504)	0.5	1	≤0.016-2	95.8	100.0	-	-
Other streptococci (500)	0.25	1	≤0.016-4	90.0	99.4	100.0	-
Enterococci (515)	2	4	0.06-8	25.6	63.9	97.7	100.0
H. influenzae (501)	2	8	0.06-32	35.9	67.3	85.6	97.0
Other organisms (68)	>32	>32	0.12->32	19.1	23.5	29.4	32.4
A. lwoffii (2)[a]	8	-	8-16	0.0	0.0	0.0	50.0
A. urinae (2)[a]	0.12	-	0.12-0.25	100.0	-	-	-
B. cereus (2)[a]	0.5	-	0.25	100.0	-	-	-
B. cepacia (2)[a]	16	-	16	0.0	0.0	0.0	0.0
G. morbillorum (2)[a]	0.25	-	0.25	100.0	-	-	-
H. parainfluenzae (2)[a]	4	-	4	0.0	0.0	100.0	-
Lactobacillus spp. (1)[a]	-	-	8	0.0	0.0	0.0	100.0
Leuconostoc spp. (1)[a]	-	-	1	100.0	-	-	-
L. monocytogenes (2)[a]	2	-	2	0.0	100.0	-	-
M. luteus (2)[a]	0.12	-	0.12	100.0	-	-	-
M. catarrhalis (2)[a]	0.25	-	0.25	100.0	-	-	-
P. multocida (2)[a]	1	-	1	100.0	-	-	-
S. maltophilia (4)[a]	4	-	2-16	0.0	25.0	75.0	75.0

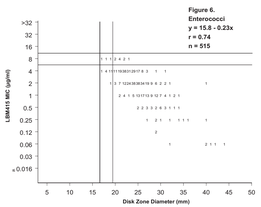
a. Only species with a LBM415 MIC at ≤ 16 µg/ml were listed among tested strains in this group (26 strains).

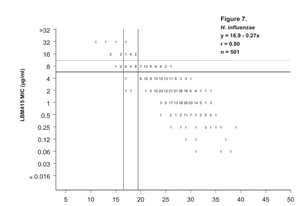


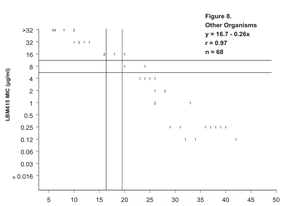












CONCLUSIONS

- The activity of LBM415 was clearly defined by both in vitro susceptibility methods demonstrating potencies versus all important, multi-drug resistant Gram-positive species and selected Gram-negative pathogens such as *Haemophilus* spp. and *M. catarrhalis*.
- The selected 30-µg disk produced reproducible zones of inhibition that correlated well (r=0.82) with MIC values across a diverse population of tested species and genera.
- Intermethod error rates ranged from 0.00% for testing streptococci, staphylococci and enterococci to 10.38% (minor) for *H. influenzae*. The very major (false-susceptible) error rate ranged from 0.00% to only 1.47% for the "other organisms" group.
- For a total of four major organism groups, no isolate was identified with MIC or zone diameter results that would indicate resistance to LBM415. For three of these organisms (*S. pneumoniae*, other streptococci, staphylococci), only a susceptible interpretive category might be considered until resistant strains are observed. However, enterococci and *H. influenzae* should have susceptible, intermediate and resistant criteria dictated by additional PK/PD and MIC population considerations.
- The absolute intermethod interpretive agreement for LBM415 tests was high and well within acceptable guidelines [NCCLS].

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