Poster
E-1180
53rd Annual ICAAC
10-13 September, 2013
Denver, CO

Comparative Analysis of Eravacycline (TP-434) by Broth Microdilution and Disk Diffusion

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Abstract

Background: Eravacycline (ERV) is ideally suited as a broad spectrum intravenous antibiotic with potential for oral step-down for empiric treatment of severe and life-threatening bacterial infections caused by the majority of multidrug-resistant Gram-negative and Gram-positive aerobes, anaerobes, and atypicals. This study evaluated the in vitro activity of ERV and comparator antimicrobials by broth microdilution and disk diffusion against recent Gram-positive and Gram-negative clinical isolates.

Methods: Bacterial isolates were mostly from Europe (52%) and North America (45.8%). Microtiter broth susceptibility and disk diffusion testing were performed using CLSI guidelines; ERV disks (20 µg) from two different manufacturers were evaluated. Scattergrams were plotted with IHMA's proprietary software.

Results: Disk diameters for the two disk lots were either identical or within ± 1 mm for 97% of the isolates. ERV was the most potent antimicrobial overall against all Gram-negative isolates (n=862 isolates), with a unimodal distribution of disk zone diameters and a modal value of 15 mm. The $MIC_{50/90}$ values (0.12/0.25 µg/mL) were 4- and 16-fold lower compared to tigecycline against 135 *Escherichia coli* isolates. A total of 99 *Klebsiella pneumoniae* isolates, including carbapenem-resistant (n=33) and cephalosporin-resistant (n=19) isolates, had MIC_{50}/MIC_{90} values of 0.25/1 µg/mL, with similar MIC values for most other *Enterobacteriaceae*. *Acinetobacter* spp. ($MIC_{50/90}$: 0.12/0.5 µg/ml) *Stenotrophomonas maltophilia* ($MIC_{50/90}$: 0.5/1 µg/ml), and respiratory pathogens *Haemophilis influenzae* ($MIC_{50/90}$: 0.12/0.25 µg/mL) and *Moraxella catarrhalis* ($MIC_{50/90}$: 0.03/0.06 µg/mL) were quite susceptible. *Pseudomonas aeruginosa* was the least susceptible ($MIC_{50/90}$: 8/16 µg/mL). ERV had an average zone diameter of 22-23 mm and a unimodal distribution of MIC values with a clear mode at 0.06 µg/mL for the Gram-positive isolates (n=579).

Conclusions: Disk zone diameter and MIC values had good correlation among the species tested with acceptable error rates observed using the epidemiological breakpoints suggested in the scattergrams. ERV appeared to have excellent activity against this diverse collection of Gram-negative and Gram-positive bacterial species.

Background

Eravacycline (formerly TP-434), has been shown to be effective against a majority of Gram-negative multidrug-resistant (MDR) pathogens in preclinical studies[1]. In phase 1 studies, eravacycline was well tolerated, demonstrating an acceptable safety profile and excellent exposure[2].

Eravacycline is ideally suited as a broad spectrum intravenous (IV) antibiotic with potential for oral step-down for empiric treatment of severe and life-threatening bacterial infections. It has the potential to be used as a once-daily IV monotherapy capable of treating MDR gram-negative pathogens and its efficacy was confirmed in recent a phase 2 trial in treatment of complicated intra-abdominal infections[3]. Eravacycline also offers potent, broad spectrum coverage of other serious and MDR gram-positive, anaerobic, and atypical pathogens.

Tetraphase is continuing to demonstrate eravacycline's differentiated profile in two phase 3 studies to assess the treatment of complicated intra-abdominal infections (cIAIs) and complicated urinary tract infections (cUTIs).

The current study aims to further determine the comparison of the activity of eravacycline when tested by broth microdilution and disk diffusion against a bank of Gram-negative and Gram-positive pathogens.

Methods

This study evaluated the in vitro activity eravacycline (TP-434) and comparator antimicrobials by broth microdilution and disk diffusion against Gram-positive and Gram-negative isolates derived from intra-abdominal infections, skin infections, pulmonary infections and urinary tract infections from 2011-2012. Wherever possible, 50% of isolates per species were from North America and 50% from Europe (Table 2). No more than one strain was isolated from any individual patient.

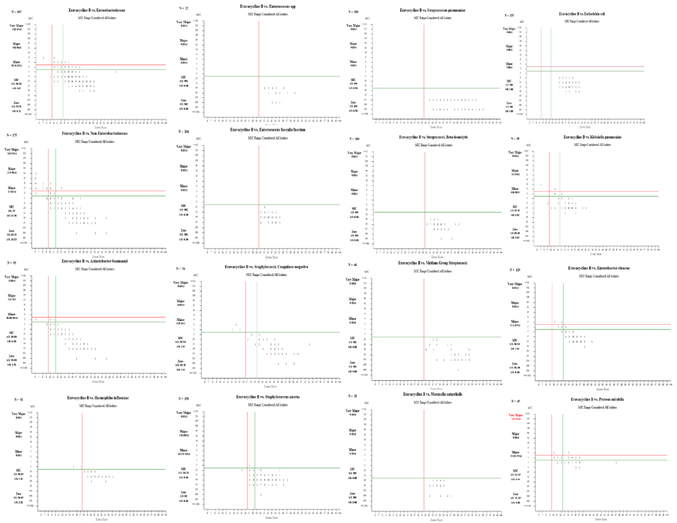
Minimum inhibitory concentration (MIC) endpoints were determined by broth microdilution according to CLSI guidelines[4]. Quality control (QC) testing was performed each day of testing as specified by the CLSI[5] using *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 49619, *Haemophilus influenzae* ATCC 49247, and *H. influenzae* ATCC 49766.

Disk diffusion testing was performed following CLSI guidelines[6] using eravacycline disks from Mast (Merseyside, UK; lot number 307781, defined as Eravacycline Disk M) and BioRad (Marnes La Coquette, France; lot number 2H0010, defined as Eravacycline Disk B). Imipenem disks (Oxoid, Basingstoke, UK; lot number 1198847) were used as a comparator for Gram-negative organisms. Linezolid disks (Mast, Merseyside, UK; lot number 1194854) were used as a comparator for Gram-positive organisms. Quality control testing was performed each day of testing as specified by the CLSI[5] using *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. All QC results were within CLSI guidelines[6], where ranges were available. The total number of isolates (n), MIC_{50} (µg/ml), MIC_{90} (µg/ml), and MIC (µg/ml) ranges were determined for all antimicrobial agents tested by species and by phenotypes.

Calculations were performed using IHMA's proprietary Surveillance Data Link Network™ software, Microsoft Excel 2007, and verified in SAS JMP version 9.0.2. Tables and graphs were exported to and formatted in Microsoft® Office Excel and Word 2007. Scattergrams were plotted with IHMA's proprietary Realtime Scattergram™ interactive software. There was a total of 1,441 isolates evaluated in this study with 607 *Enterobacteriaceae* (11 species), 176 non-fermentative gram-negative bacilli (five species), 150 *S. aureus,* 54 coagulase-negative *Staphylococcus* spp. and 129 *Enterococcus* spp.. Fastidious species included *H. influenzae* (51 isolates; 21.6% β-lactamase positive), *M. catarrhalis* (28 isolates), β-haemolytic *Streptococcus* spp. (100 isolates: 3 species groups), *S. pneumoniae* (100 isolates) and viridans group *Streptococcus* spp. (46 isolates from multiple species groups) (Table 1). Demographically, the isolates were distributed regionally among Europe, 52.2%, North America, 45.8%, and much smaller numbers from Asia/Pacific, 0.3% and Latin America, 1.7% (Table 2).

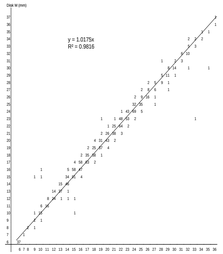
Results

Figure 1. Disk versus Broth Scattergrams for Eravacycline



Disk versus broth MIC correlation scattergrams for eravacycline against major pathogen groups were plotted with IHMA's proprietary Realtime Scattergram™ interactive software.

Figure 2. Correlation of Zone Size per Isolate Between Manufacturers



Analysis of zone sizes for eravacycline disks from two manufacturing centers. Eravacycline disk M refers to Mast and Eravacycline Disk B refers to BioRad. There appears no observable differences between manufacturers with only a few outliers observed graphically. The overall regression statistics suggest there is no difference between lot B and lot M (R^2 = 0.9816). Analysis shows that >59% of the disk values were identical between lots, 97% were within +/-1 mm between lots and that slightly lower zone diameters were obtained with disk M versus disk B.

Table 1. Eravacycline $MIC_{50/90}$ Values for Study Organisms

Organism Name	N	% of total N	MIC 50/90 (µg/ml)
Enterobacteriaceae	**607**	**42.10%**	
Citrobacter freundii	14	1.00%	0.25/0.5
Citrobacter koseri	2	0.10%	n/a
Enterobacter aerogenes	47	3.30%	0.25/1
Enterobacter cloacae	129	9.00%	0.5/1
Escherichia coli	135	9.40%	0.12/0.25
Escherichia coli, Carbapenem-Res	4	0.30%	n/a
Escherichia coli, Cephalosporin-Res	12	0.80%	0.25/0.25
Klebsiella pneumoniae	99	6.90%	0.25/1
Klebsiella pneumoniae, Carbapenem-Res	33	2.30%	1/2
Klebsiella pneumoniae, Cephalosporin-Res	19	1.30%	0.5/1
Morganella morganii	9	0.60%	n/a
Proteus mirabilis	45	3.10%	1/2
Proteus vulgaris	1	0.10%	n/a
Providencia stuartii	1	0.10%	n/a
Serratia marcescens	57	4.00%	1/2
Enterococci	**129**	**9.00%**	
Enterococcus spp:	25	1.70%	0.03/0.06
Enterococcus avium	8	0.60%	n/a
Enterococcus casseliflavus	8	0.60%	n/a
Enterococcus durans	4	0.30%	n/a
Enterococcus gallinarum	1	0.10%	n/a
Enterococcus raffinosus	4	0.30%	n/a
Enterococcus faecalis, VRE	21	1.50%	0.06/0.12
Enterococcus faecalis, VSE	30	2.10%	0.06/0.12
Enterococcus faecium, VRE	24	1.70%	0.06/0.06
Enterococcus faecium, VSE	29	2.00%	0.03/0.06
Haemophilus influenzae	**51**	**3.50%**	
Haemophilus influenzae, BL-Neg	40	2.80%	0.12/0.25
Haemophilus influenzae, BL-Pos	11	0.80%	0.12/0.25
Moraxella catarrhalis	**28**	**1.90%**	
Moraxella catarrhalis	28	1.90%	0.03/0.06
Non-Enterobacteriaceae	**176**	**12.20%**	
Acinetobacter baumannii	95	6.60%	0.12/1
Acinetobacter calcoaceticus	1	0.10%	n/a
Acinetobacter lwoffii	5	0.30%	n/a
Pseudomonas aeruginosa	50	3.50%	8/16
Stenotrophomonas maltophilia	25	1.70%	0.5/1
Staphylococci, Coagulase-negative	**54**	**3.70%**	
Staphylococci, Coagulase-negative, MR	19	1.30%	0.12/2
Staphylococci, Coagulase-negative, MS	35	2.40%	0.06/0.25
Staphylococcus aureus	**150**	**10.40%**	
Staphylococcus aureus, MRSA	82	5.70%	0.06/0.12
Staphylococcus aureus, MSSA	68	4.70%	0.06/0.12
Streptococci, Beta-hemolytic	**100**	**6.90%**	
Streptococcus agalactiae	77	5.30%	0.03/0.03
Streptococcus pyogenes	20	1.40%	0.015/0.03
Streptococcus, Beta-H, Grp C	3	0.20%	n/a
Streptococcus pneumoniae	**100**	**6.90%**	
Streptococcus pneumoniae	100	6.90%	≤0.008/0.015
Viridans Group Streptococci	**46**	**3.20%**	
Streptococcus anginosus	1	0.10%	n/a
Streptococcus bovis	1	0.10%	n/a
Streptococcus gallolyticus	1	0.10%	n/a
Streptococcus mitis	1	0.10%	n/a
Streptococcus salivarius	1	0.10%	n/a
Viridans group streptococci, unspeciated	41	1.60%	0.03/0.25
Total	**1441**	**100.00%**	

Eravacycline $MIC_{50/90}$ values calculated from broth microdilution assays. Values were not calculated for any group where number of isolates were < 10

Table 2. Geographical Distribution of Study Organisms

Country	Asia	Europe	Latin American	North America	Total N	% of Total N by Country
Argentina			5		5	0.30%
Austria		14			14	1.00%
Belgium		31			31	2.20%
Brazil			3		3	0.20%
Bulgaria		5			5	0.30%
Canada				18	18	1.20%
Chile			5		5	0.30%
China	5				5	0.30%
Colombia			9		9	0.60%
Croatia		13			13	0.90%
Czech Republic		12			12	0.80%
Denmark		9			9	0.60%
Estonia		1			1	0.10%
Finland		3			3	0.20%
France		93			93	6.50%
Germany		93			93	6.50%
Greece		26			26	1.80%
Hungary		14			14	1.00%
Italy		102			102	7.10%
Latvia		33			33	2.30%
Lithuania		3			3	0.20%
Mexico			2		2	0.10%
Netherlands		9			9	0.60%
Portugal		23			23	1.60%
Puerto Rico				6	6	0.40%
Romania		18			18	1.20%
Serbia		7			7	0.50%
Slovak Republic		3			3	0.20%
Slovenia		5			5	0.30%
Spain		152			152	10.50%
Sweden		23			23	1.60%
Switzerland		9			9	0.60%
Turkey		19			19	1.30%
United Kingdom		32			32	2.20%
United States				636	636	44.10%
Total N by Region	**5**	**752**	**24**	**660**	**1441**	**100%**
% of Total N by Region	**0.30%**	**52.20%**	**1.70%**	**45.80%**	**100%**	

Geographical location data for all organisms used in this study. A total of 52.2% of isolates originated in Europe while 45.8% were from North America. All isolates were pulled from IHMA frozen storage and were derived from intra-abdominal infections, skin infections, pulmonary infections and urinary tract infections from 2011-2012. No more than one strain was isolated from any individual patient.

Conclusions

- Eravacycline appeared to have excellent activity against this diverse collection of Gram-negative and Gram-positive bacterial species. Limited activity was observed for eravacycline against *P. aeruginosa.*
- A comparison of zone diameters for the two lots of eravacycline disks found values between the two lots either identical or within ± 1 mm for 97% of the isolates. Thus, disk load can be adequately reproduced by two different manufacturers.
- Disk zone diameter and MIC values had good correlation among the species tested in this study with acceptable error rates observed using the epidemiological breakpoints suggested in the scattergrams.
- ≥82% of the isolates that would be considered to be susceptible by the microtiter broth dilution or disk analyses are within the epidemiological breakpoints established in this study for *Enterobacteriaceae, H. influenzae*, *Enterococcus* spp., coagulase-negative staphylococci, *S. aureus, S. pneumoniae*, β-hemolytic streptococci, viridans group streptococci, and *M. catarrhalis*. However, only 72.0% and 61.7% of the non-*Enterobacteriaceae* and 96.8% and 76.8% of the *A. baumannii* isolates were within the proposed interpretative criteria for MIC and disk diffusion, respectively.

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Acknowledgements: This project has been funded in whole or in part with Federal funds from the Biomedical Advanced Research and Development Authority, Office of the Assistant Secretary for Preparedness and Response, Office of the Secretary, Department of Health and Human Services, under Contract No HHSO100201200002C.

Authors would like to thank Corey Fyfe, Tetraphase Pharmaceuticals, Inc. for his efforts on poster design.