#E-328

Recently Collected Extended Spectrum Beta-Lactamase (ESBL) Isolates (354) Evaluated In Vitro Against Tigecycline and Comparator Agents: Global Perspective

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REVISED ABSTRACT

Background: Tigecycline (TIG), a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent expanded broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections. The T.E.S.T. program determined the in vitro activity of TIG compared to amoxicillin-clavulanic acid, piperacillin-tazobactam (PT), levofloxacin, ceftriaxone, cefepime, ampicillin (AMP), amikacin (AK), minocycline, ceftazidime and imipenem (IMP) against ESBL isolates collected from hospitals globally throughout 2004-2005. **Methods**: A total of 354 ESBL-producing clinical isolates were identified to the species level from participating site and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using supplied broth microdilution panels and interpreted according to CLSI guidelines, except for tigecycline, which used the susceptible breakpoint of $\leq$2 mcg/mL for *Enterobacteriaceae* as defined in the FDA package insert. **Results**: %S for all ESBL-producing isolates vs. TIG, IMP, and AK was 93.2, 91.0, and 90.7%, respectively; %S for other comparators ranged from a high of 66.9% (PT) to a low of 0.3% (AMP). $MIC_{50}/_{90}$ for TIG, IMP, and AK were 0.5/2, 0.5/2, and 4/16 mcg/mL; the MIC_{90} for all other drugs was in the resistant range. There were minor regional differences in levels of activity, with either TIG (North America) or IMP (Europe, Asia/Pac) being the most active compound in this study. **Conclusions**: TIG is as active in vitro against ESBL strains as IMP. Its expanded broad spectrum of activity, including Gram-negative and -positive strains resistant or multiply-resistant to other agents, should make it a very useful treatment option for difficult to treat ESBL producing *Enterobacteriaceae*.

INTRODUCTION

Tigecycline (formerly GAR-936) is a member of a new class of antimicrobial agents, the glycylcyclines. This synthetic analogue of the tetracyclines exhibits significant antibacterial activity that is both bacteriostatic and, in certain instances, bactericidal with killing activity that is as much as fourfold better than vancomycin and daptomycin [1, 2]. The development of tigecycline is important in that it and other glycylcyclines are active against bacterial strains carrying either or both of the two major forms of tetracycline resistance: efflux and ribosomal protection. Certain substituents at the 9-position of the tetracycline molecule restored activity against bacteria harboring genes encoding either or both efflux and ribosomal protection. A single chemical modification of tigecycline overcomes the two molecularly distinct forms of resistance while maintaining activity against susceptible gram-positive, gram-negative, aerobic, and anaerobic bacteria [3]. Furthermore, resistance to tigecycline is difficult to produce even in the laboratory.

Previous studies have demonstrated excellent in vitro activity for tigecycline against clinical and laboratory strains of Gram-positive and -negative bacteria with MIC_{90}s at or below 2 mcg/mL, including difficult-to-treat methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant enterococci (VRE) and extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae [4-7]. The relatively limited treatment options for infections due to ESBL-producing strains in particular have led to a need for new compounds with activity against these problematic pathogens. This study was undertaken to document the in vitro activity of tigecycline against a significant number of clinical isolates of ESBL-producing Enterobacteriaceae from North America, Europe, Middle East, Latin America and Asia/Pacific. This study is part of the larger ongoing global Tigecycline Evaluation and Surveillance Trials (T.E.S.T.) program.

MATERIALS & METHODS

- All isolates were derived from blood, respiratory tract, urine, skin, wound, body fluids and other defined sources. Only one isolate per patient was accepted into the study. Clinical isolates were collected and tested between January 2004 - July 2005 from 107 sites in 25 countries in North America, Europe, Middle East, Latin America and Asia/Pacific. Isolates were identified to the species level and tested at each site by the participating laboratory.
- Organism collection, transport, confirmation of organism identification, and development and management of a centralized database were coordinated by Laboratories International for Microbiology Studies (LIMS), a division of International Health Management Associates, Inc. located in Schaumburg, IL, USA.
- All organisms were deemed clinically significant by local participant criteria. Isolate inclusion was independent of medical history, antimicrobial use, age or gender. All sites identified each study isolate utilizing local laboratory site criteria.

Antimicrobial Susceptibility Testing

- Minimum inhibitory concentrations (MICs) were determined by the CLSI recommended broth microdilution testing method [8]. Tigecycline was supplied by Wyeth Pharmaceuticals (Collegeville, PA, USA). All other agents were supplied by the panel manufacturer, MicroScan (Dade Behring Inc., West Sacramento, CA, USA). The following antimicrobial agents and dilution ranges (expressed in mcg/mL) were included on the panels: tigecycline (0.008-16), amoxicillin/clavulanic acid (0.12/0.06-32/16), ampicillin (0.5-32), imipenem (0.06-16), levofloxacin (0.008-8), minocycline (0.5-16), piperacillin/tazobactam (0.06/4-128/4), amikacin (0.5-32), ceftazidime (8-32), ceftriaxone (0.06-64), and cefepime (0.5-32). MIC interpretive criteria followed published guidelines established by the Clinical and Laboratory Standards Institute [9] and the recent US Food and Drug Administration package insert for tigecycline [10], where applicable.
- Quality controls (QC) were performed by each testing site on each day of testing using the corresponding ATCC control strains: *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853. Results were included in the analysis only when corresponding QC isolates tested within the acceptable range according to CLSI (2005) guidelines [9].

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ACKNOWLEDGEMENTS

We gratefully acknowledge the contributions of the investigators, laboratory personnel and all members of the Tigecycline Evaluation Study Trials program group. This study was sponsored by a grant from Wyeth Pharmaceuticals.

RESULTS

Results are shown in the following tables.

Table 1. In vitro activity of tigecycline and comparative antimicrobial agents against 354 extended spectrum beta-lactamase producing isolates from a global population

Organism (N)	Drug	MIC (mcg/mL) MIC_{50}	MIC_{90}	Range	%Sus [a]
All ESBL producers (n=354)	Tigecycline	0.5	2	0.06 - 8	93.2
	Amikacin	4	16	0.5 - 32	90.7
	AmoxClav	16	>32	2 - >32	27.4
	Ampicillin	>32	>32	4 - >32	0.3
	Cefepime	8	>32	0.5 - >32	50.8
	Ceftazidime	>32	>32	8 - >32	21.5
	Ceftriaxone	64	>64	0.06 - >64	20.3
	Imipenem	0.5	2	0.12 - >16	91
	Levofloxacin	8	>8	0.015 - >8	35.9
	Minocycline	4	>16	0.5 - >16	66.1
	Pip-tazo	8	128	0.5 - 128	66.9
E. coli (n=103)	Tigecycline	0.25	0.5	0.06 - 4	99
	Amikacin	4	8	1 - 32	99
	AmoxClav	16	>32	4 - >32	21.4
	Ampicillin	>32	>32	4 - >32	1
	Cefepime	16	>32	$\leq$0.5 - >32	41.7
	Ceftazidime	16	>32	$\leq$8 - >32	36.9
	Ceftriaxone	>64	>64	$\leq$0.06 - >64	14.6
	Imipenem	0.25	0.5	0.12 - 2	100
	Levofloxacin	>8	>8	0.015 - >8	19.4
	Minocycline	4	>16	$\leq$0.5 - >16	62.1
	Pip-tazo	4	64	0.5 - >128	88.3
K. pneumoniae (n=230)	Tigecycline	1	4	0.12 - 8	90
	Amikacin	4	32	$\leq$0.5 - 32	87
	AmoxClav	16	>32	2 - >32	27.8
	Ampicillin	>32	>32	16 - >32	0
	Cefepime	8	>32	$\leq$0.5 - >32	52.6
	Ceftazidime	>32	>32	$\leq$8 - >32	14.8
	Ceftriaxone	64	>64	0.12 - >64	22.6
	Imipenem	0.5	8	0.25 - >16	86.1
	Levofloxacin	8	>8	0.03 - >8	39.6
	Minocycline	4	>16	1 - >16	67.4
	Pip-tazo	16	>128	1 - >128	56.5
K. oxytoca (n=21)	Tigecycline	0.5	1	0.25 - 2	100
	Amikacin	4	16	1 - 32	90.5
	AmoxClav	8	32	2 - 32	52.4
	Ampicillin	>32	>32	>32 - >32	0
	Cefepime	4	>32	$\leq$0.5 - >32	76.2
	Ceftazidime	>32	>32	$\leq$8 - >32	19
	Ceftriaxone	16	64	0.12 - >64	23.8
	Imipenem	0.5	0.5	0.25 - 1	100
	Levofloxacin	1	4	0.03 - >8	76.2
	Minocycline	4	16	$\leq$0.5 - >16	71.4
	Pip-tazo	4	>128	0.5 - >128	76.2

[a]Interpretive criteria as defined by CLSI, M100-S15 (2005); tigecycline breakpoints are according to FDA package insert (2005) [10]

Table 2. Regional susceptibility results for 354 ESBL-producing *Enterobacteriaceae*

Region	Drug	%Sus[a]	%Int[a]	%Res[a]
North America (n=147)	Tigecycline	93.2	5.4	1.4
	Amikacin	85	15	0
	AmoxClav	29.3	27.9	42.9
	Ampicillin	0	1.4	98.6
	Cefepime	53.7	6.8	39.5
	Ceftazidime	9.5	4.8	85.7
	Ceftriaxone	26.5	24.5	49
	Imipenem	78.2	14.3	7.5
	Levofloxacin	25.2	5.4	69.4
	Minocycline	70.7	8.8	20.4
	Pip-tazo	52.4	4.1	43.5
Europe (n=125)	Tigecycline	89.6	9.6	0.8
	Amikacin	95.2	4.8	0
	AmoxClav	32.8	40.8	26.4
	Ampicillin	0.8	0	99.2
	Cefepime	54.4	11.2	34.4
	Ceftazidime	30.4	8.8	60.8
	Ceftriaxone	22.4	22.4	55.2
	Imipenem	100	0	0
	Levofloxacin	41.6	13.6	44.8
	Minocycline	57.6	13.6	28.8
	Pip-tazo	73.6	8	18.4
Asia/Pacific (n=45)	Tigecycline	97.8	2.2	0
	Amikacin	100	0	0
	AmoxClav	26.7	60	13.3
	Ampicillin	0	0	100
	Cefepime	57.8	15.6	26.7
	Ceftazidime	40	17.8	42.2
	Ceftriaxone	11.1	15.6	73.3
	Imipenem	100	0	0
	Levofloxacin	46.7	2.2	51.1
	Minocycline	75.6	13.3	11.1
	Pip-tazo	93.3	4.4	2.2
Middle East (n=24)	Tigecycline	100	0	0
	Amikacin	91.7	8.3	0
	AmoxClav	0	0	100
	Ampicillin	0	0	100
	Cefepime	12.5	4.2	83.3
	Ceftazidime	16.7	12.5	70.8
	Ceftriaxone	0	12.5	87.5
	Imipenem	100	0	0
	Levofloxacin	41.7	0	58.3
	Minocycline	58.3	20.8	20.8
	Pip-tazo	75	8.3	16.7
Latin America (n=13)	Tigecycline	100	0	0
	Amikacin	76.9	23.1	0
	AmoxClav	7.7	15.4	76.9
	Ampicillin	0	0	100
	Cefepime	30.8	23.1	46.2
	Ceftazidime	15.4	7.7	76.9
	Ceftriaxone	0	15.4	84.6
	Imipenem	100	0	0
	Levofloxacin	53.8	7.7	38.5
	Minocycline	76.9	23.1	0
	Pip-tazo	61.5	0	38.5

[a]Interpretive criteria as defined by CLSI, M100-S15 (2005); tigecycline breakpoints are according to FDA package insert (2005) [10]

Table 3. Frequency distribution and cumulative % inhibition for tigecycline and comparators vs. 32 isolates of imipenem-resistant ESBL producing *Klebsiella pneumoniae*

	MIC (mcg/mL)													
N/Cum%	0.25	1	2	4	8	>8	16	>16	32	>32	64	>64	128	>128
Tigecycline	2 6.3	23 78.1	5 93.8	2 100										
Amikacin			2 6.3	2 12.5	4 25		11 59.4		13 100					
AmoxClav										32 100				
Ampicillin										32 100				
Cefepime							1 3.1		4 15.6	27 100				
Ceftazidime										32 100				
Ceftriaxone									1 3.1		4 15.6	27 100		
Imipenem					21 65.6		9 93.8	2 100						
Levofloxacin					1 3.1	31 100								
Minocycline		1 3.1	6 21.9	18 78.1	5 93.8		2 100							
Pip-tazo													1 3.1	31 100

[a] MIC in mcg/mL
[b] Interpretive criteria as defined by CLSI, M100-S15 (2005), where available; Tigecycline susceptible breakpoint is according to FDA package insert (2005) where available [9]; na = not available.
[c] Extended Spectrum Beta-Lactamase producing strains
[d] MIC_{50} and MIC_{90} values not calculated for species or phenotypic groups with n < 10. Species with aggregate n's < 20 are not shown.

CONCLUSIONS

- Tigecycline was the most active compound vs. ESBL-producing *E. coli*, *K. pneumoniae*, and *K. oxytoca*, inhibiting 93.2% of the strains, whereas imipenem and amikacin inhibited 91.0% and 90.7%, respectively.
- The regional differences in levels of imipenem activity (78.2% susceptible in North America, but 100% susceptible in all other areas) were due to 32 imipenem-non-susceptible strains of *K. pneumoniae* from 7 hospitals located within a 100 mile radius of New York City. These results corroborate recent reports of an outbreak of carbapenem-resistant *K. pneumoniae* (mediated by KPC beta-lactamases) in the New York area [11-13]. Bratu et al [13] reported that 100% (96/96) of these imipenem non-susceptible *K. pneumoniae* were susceptible to tigecycline; in the present study, 93.8% were susceptible to tigecycline, and the remaining 6.2% (2/32 strains) were "intermediate", with MICs of 4 mcg/mL. None of the imipenem-resistant strains in this study was resistant to tigecycline.
- Tigecycline appears to be a valid alternative to imipenem for therapy of serious infections caused by ESBL-producing *E. coli*, *K. pneumoniae*, or *K. oxytoca*; furthermore, it is one of the only presently-available antimicrobics effective against KPC-possessing carbapenem-resistant *K. pneumoniae*.