The Efficacy of Tigecycline Against AmpC Producing Enterobacter spp. and Serratia marcescens

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REVISED ABSTRACT

Objectives: Beta-lactamase production is the most common cause of beta-lactam antibiotic resistance in gram-negative bacteria. AmpC beta-lactamases, presumably chromosomally mediated, are associated with multi-drug resistance in both *Enterobacter* spp. and *Serratia* spp. The emergence of these difficult to treat strains has severely reduced therapeutic options and resulted in treatment failures. Tigecycline, a new glycylcycline, offers the potential of enhanced activity against AmpC producing strains. The Tigecycline Evaluation Surveillance Trial (T.E.S.T.) evaluated the activity of tigecycline and comparators against AmpC producing *Serratia marcescens* and *Enterobacter* spp. from a global population. **Methods:** 862 clinically significant isolates with the AmpC phenotype were collected between 2004 and 2007. MICs were determined at each site using common broth microdilution panels and results interpreted as specified by CLSI at each site. Additional testing was performed by Laboratories International for Microbiology Studies (LIMS), a subsidiary of International Health Management Associates, Inc. (IHMA, Schaumburg, IL, USA). **Results:** $MIC_{50/90}$ (mcg/mL) and % susceptible (S) of tigecycline and comparators are shown in the following table:

Drug	*Enterobacter* spp. (n=821)			*Serratia marcescens* (n=41)		
	%S	MIC_{50}	MIC_{90}	%s	MIC_{50}	MIC_{90}
Tigecycline	87.6	0.5	4	90.2	1	2
Amikacin	95.5	2	8	85.4	4	32
AmoxClav	0.7	>32	>32	0	>32	>32
Cefepime	100	4	8	100	4	8
Ceftazidime	0	>32	>32	0	>32	>32
Ceftriaxone	0	64	>64	0	64	>64
Imipenem	99.6	0.5	1	100	1	1

Conclusion: Tigecycline is a promising expanded broad spectrum antibiotic showing significant activity against AmpC producing *Enterobacter* spp. and *Serratia marcescens* from a world wide population. With MIC_{50} values of 0.5 mcg/mL versus *Enterobacter* and 1 mcg/mL versus *Serratia*, this study validates tigecycline's potent inhibitory activity against these pathogens.

INTRODUCTION

Beta-lactamase synthesis is the most common mechanism for resistance to beta-lactam antibiotics in gram-negative bacteria (1). High level production of inducible chromosomal AmpC beta-lactamase by *Serratia marsescens* and *Enterobacter* spp. provides resistance to both oxyimino- and 7- alpha - methoxy-cephalosporins and monobactams (2). 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 [3, 4]. The development of tigecycline is important in that tigecycline 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 restore 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 [5]. 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 gram-negative bacteria with minimum inhibitory concentrations for the 90th percentile inhibited 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* [6-8]. This study was undertaken to document the in vitro activity of tigecycline against *Serratia marsescens* and *Enterobacter* spp. exhibiting the AmpC beta-lactamase phenotype. This study is part of the ongoing global Tigecycline Evaluation and Surveillance Trial (T.E.S.T.) program.

MATERIALS & METHODS

- All isolates were derived from blood, respiratory tract, urine (no more than 25% of all isolates), skin, wound, body fluids, and other defined sources. Only one isolate per patient was accepted into the study. Over 150,470 clinical isolates were collected and tested between 2004 and 2007 from 387 investigative sites in 48 countries worldwide. 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 criteria.
- Minimum inhibitory concentrations (MICs) were determined by the CLSI recommended broth microdilution testing method [9]. Tigecycline was supplied by Wyeth Pharmaceuticals (Collegeville, PA, USA). All other agents were supplied by the panel manufacturer, MicroScan (Dade Behring Inc., Sacramento, CA, USA) and Trek (TREK Diagnostic Systems, Cleveland, OH). The following antimicrobial agents were included on the panels with their dilution ranges (expressed in mcg/ml): amikacin (0.5-64); amoxicillin/clavulanic acid (0.12/0.06-32/16, tested using a 2:1 ratio of amoxicillin:clavulanic acid; reported concentrations refer to amoxicillin); ampicillin (0.5-32); cefepime (0.5-32); ceftriaxone (0.06-64); ceftazidime (8-32); imipenem (0.06-16); levofloxacin (0.008-8); minocycline (0.5-16); tigecycline (0.008-16) and piperacillin/tazobactam (0.06/4-128/4). MIC interpretive criteria followed published guidelines established by the Clinical and Laboratory Standards Institute [10] and the recent US Food and Drug Administration package insert for tigecycline [11], where applicable.
- Quality controls (QC) were performed by each testing site on each day of testing using the corresponding ATCC control strains: *E. coli* ATCC 25922; *Pseudomonas aeruginosa* ATCC 27853. Results were included in the analysis only when corresponding QC isolates tested within the acceptable range according to CLSI (2007) guidelines [10].

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RESULTS

Results are shown in the following tables.

Table 1. In vitro activity (mcg/mL) of tigecycline and comparators against 821 *Enterobacter* spp. with the AmpC phenotype.

Organism (n)	Drug	%Sus	%Int	%Res	MIC (mcg/mL) MIC_{50}	MIC_{90}
Enterobacter spp.	Tigecycline	87.6	7.9	4.5	0.5	4
(n=821)	Amikacin	95.5	1.6	2.9	2	8
	Amox-Clav	0.7	1.2	98.1	>32	>32
	Ampicillin	0	0	100	>32	>32
	Cefepime	100	0	0	4	8
	Ceftazidime	0	0	100	>32	>32
	Ceftriaxone	0	0	100	64	>64
	Imipenem	99.6	0.2	0.2	0.5	1
	Levofloxacin	64.8	5.7	29.5	0.5	>8
	Minocycline	58.8	18.9	22.3	4	>16
	PipTazo	8	37.5	54.4	128	>128

Table 2. In vitro activity of tigecycline and comparators against 821 *Enterobacter* spp. with the AmpC phenotype showing cumulative percent inhibited (%) at each MIC (mcg/ml).

n/Cum%	MIC (mcg/mL)* 0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128
Tigecycline			1	5	88	317	188	120	65	37				
			0.1	0.7	11.4	50.1	73	87.6	95.5	100				
Amikacin						18	331	270	61	64	40	13	9	15
						2.2	42.5	75.4	82.8	90.6	95.5	97.1	98.2	100
Amox-Clav										6	10	53	752	
										0.7	1.9	8.4	100	
Ampicillin												4	817	
												0.5	100	
Cefepime						24	53	155	321	268				
						2.9	9.4	28.3	67.4	100				
Ceftazidime												109	712	
												13.3	100	
Ceftriaxone													430	391
													52.4	100
Imipenem			12	46	162	267	59	9	5	1		1		
			2.1	10.3	39.1	86.7	97.2	98.8	99.6	99.8		100		
Levofloxacin	8	155	149	34	45	59	40	42	47	47	195			
	1	19.9	38	42.1	47.6	54.8	59.7	64.8	70.5	76.2	100			
Minocycline						4	22	197	260	155	88	95		
						0.5	3.2	27.2	58.8	77.7	88.4	100		
PipTazo			1		1	2	4	4	16	13	25	100	208	447
			0.1		0.2	0.5	1	1.5	3.4	5	8	20.2	45.6	100

*Some ≤ and > values have been normalized by rounding up to the next highest MIC

Table 3. In vitro activity (mcg/mL) of tigecycline and comparators against 41 *Serratia marsescens* with the AmpC phenotype.

Organism (n)	Drug	%Sus	%Int	%Res	MIC (mcg/mL) MIC_{50}	MIC_{90}
Serratia marsescens	Tigecycline	90.2	7.3	2.4	1	2
(n=41)	Amikacin	85.4	9.8	4.9	4	32
	Amox-Clav	0	0	100	>32	>32
	Ampicillin	0	0	100	>32	>32
	Cefepime	100	0	0	4	8
	Ceftazidime	0	0	100	>32	>32
	Ceftriaxone	0	0	100	64	>64
	Imipenem	100	0	0	1	1
	Levofloxacin	78	12.2	9.8	1	4
	Minocycline	82.9	9.8	7.3	2	8
	PipTazo	46.3	26.8	26.8	32	>128

Table 4. In vitro activity of tigecycline and comparators against 41 *Serratia marsescens* with the AmpC phenotype showing cumulative percent inhibited (%) at each MIC (mcg/ml).

n/Cum%	MIC (mcg/mL)* 0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128
Tigecycline	3				2	11	18	3	3	1				
	7.3				12.2	39	82.9	90.2	97.6	100				
Amikacin							5	9	10	5	6	4	2	
							12.2	34.1	58.5	70.7	85.4	95.1	100	
Amox-Clav												3	38	
												7.3	100	
Ampicillin												1	40	
												2.4	100	
Cefepime						4		5	12	20				
						9.8		22	51.2	100				
Ceftazidime												4	37	
												9.8	100	
Ceftriaxone													24	17
													58.5	100
Imipenem				5			6	14	1	1				
				18.5			40.7	92.6	96.3	100				
Levofloxacin			4	6	1	3	11	7	5	1	3			
			9.8	24.4	26.8	34.1	61	78	90.2	92.7	100			
Minocycline						1	4	16	13	4	1	2		
						2.4	12.2	51.2	82.9	92.7	95.1	100		
PipTazo			1			1	8	2	1	2	4	4	7	11
			2.4			4.9	24.4	29.3	31.7	36.6	46.3	56.1	73.2	100

*Some ≤ and > values have been normalized by rounding up to the next highest MIC.

CONCLUSIONS

- Tigecycline exhibits excellent activity against *Serratia marsescens* and *Enterobacter* spp. with the AmpC beta-lactamase phenotype, with MIC_{50} values of 0.5 mcg/mL and 1 mcg/mL, respectively.
- Tigecycline %s susceptible for *Enterobacter* spp.(87.6%) and *Serratia marsescens* (90.2%) were comparable to those of amikacin and imipenem.
- Tigecycline's potent activity against organisms with the AmpC phenotype should make it a useful addition to the list of antimicrobial agents with clinical potential against these strains with limited therapeutic options.