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Evaluating Antibiotic Cross-Resistance in Clinical Isolates of Enterobacteriaceae from the United States against 10 Antimicrobial Agents - The T.E.S.T. Program

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REVISED ABSTRACT

Background: Tigecycline (TIG), a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent broad spectrum activity against most commonly encountered species responsible for hospital acquired infections. Cross-resistance to several classes of antimicrobials is often seen in nosocomial pathogens. The T.E.S.T. program determined the in vitro activity of tigecycline against strains of *Enterobacteriaceae* cross-resistant to one or more of the following antimicrobials: amoxicillin-clavulanic acid(AC), piperacillin-tazobactam(PT), levofloxacin(LV), ceftriaxone(CX), cefepime(CP), ampicillin (AMP), amikacin(AK), minocycline(MN), ceftazidime(CZ) and imipenem(IMP). The isolates were collected from 77 investigational sties in the United States during 2004-2005. **Methods:** A total of 851 clinical isolates cross-resistant to two or more drug classes were identified to the species level at each site and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using broth microdilution panels. Antimicrobial resistance was interpreted according to CLSI breakpoints with TIG susceptible and resistant breakpoints defined as $\leq$2 mcg/mL and $\geq$8 mcg/mL, respectively (FDA, 2005). **Results:** Resistance rates to the comparator drugs against these cross-resistant strains were CAX 24%, CAZ 38%. LVX 71%, CPE 14%, PT 23%, AK 0.1%, IMP 7%, and MIN 46%. Strains were grouped by presence of resistance to 0, 1, 2, 3 or $\geq$4 drug classes. The percentage of strains falling into Groups 0 through 4 were 20%, 65%, 12%, 3% and 0.1%, respectively. TIG inhibited $\geq$82% of all 851 multi-drug resistant strains at 2 mcg/mL. TIG $MIC_{50/90}$ for Groups 0-4 were 0.12/0.5, 0.5/1, 0.5/4, 1/4 and 2/8 mcg/mL, respectively. **Conclusion:** The presented data suggest that tigecycline is affected minimally by this cross-resistance phenomenon with MIC values staying within therapeutic range and may be an effective and reliable option against these frequent nosocomial or community pathogens regardless to the resistance patterns.

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 four fold 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.

Infections secondary *Enterobacteriaceae* present a challenge to clinicians with increasing multi-drug resistance worldwide. Resistance of *Enterobacteriaceae* to cephalosporins, and ciprofloxacin is now widespread but carbapenems, colistin, sulbactam and minocycline remain effective in over 80% of most strains [4-6]. The emergence of imipenem resistance in this species is of considerable concern leaving relatively limited treatment options for infections due these resistant *Enterobacteriaceae* and has led to a search for new compounds with activity against these problematic pathogens.

This study was undertaken to document the in vitro activity of tigecycline against multi-drug resistant *Enterobacteriaceae* from a large diverse population within the United States. 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 - October 2005 from 77 sites in the United States. 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 [7]. 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), 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 [8] and the recent US Food and Drug Administration package insert for tigecycline [9], 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 [8].

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RESULTS

Results are shown in the following tables.

Table 1. In Vitro Activity of Tigecycline and Comparators against 851 Multi-Drug Resistant* *Enterobacteriaceae* from the United States.

Drug	%Sus[a]	%Int	%Res	MIC (mcg/mL) MIC_{50}	MIC_{90}
Tigecycline	82.4	12.8	4.8	0.5	4
Amikacin	95.1	4.8	0.1	2	16
AmoxClav	30.8	17.5	51.7	32	>32
Ampicillin	0.7	0.5	98.8	>32	>32
Cefepime	82.1	4.3	13.5	$\leq$0.5	>32
Ceftazidime	57.7	4.5	37.8	$\leq$8	>32
Ceftriaxone	63.6	12.9	23.5	0.5	>64
Imipenem	89.4	4.1	6.5	0.5	8
Levofloxacin	25.9	3.3	70.9	8	>8
Minocycline	44.5	10	45.5	8	>16
PipTazo	67.1	10.2	22.7	4	>128

[a]Interpretive criteria as defined by CLSI, M100-S15 (2005); tigecycline breakpoints (in terms of mcg/mL) are susceptible $\leq$2, intermediate = 4 and $\geq$8 (Tygacil®, 2005).

Table 2. Frequency Distribution and Cumulative Percent Inhibited (%) at each MIC (mcg/mL) for Tigecycline and Comparators for 851 Multi-Drug Resistant* *Enterobacteriaceae* Isolates from the United States.

	MIC (mcg/mL)														
n/cum%	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
Tigecycline			19	176	168	117	135	86	109	41					
			2.2	22.9	42.7	56.4	72.3	82.4	95.2	100					
Amikacin						10	165	331	154	93	56	41		1	
						1.2	20.6	59.5	77.6	88.5	95.1	99.9		100	
AmoxClav								26	55	181	149	122	318		
								3.1	9.5	30.8	48.3	62.6	100		
Ampicillin									3	3	4	40	801		
									0.4	0.7	1.2	5.9	100		
Cefepime						466	62	59	56	56	37	24	91		
						54.8	62	69	75.6	82.1	86.5	89.3	100		
Ceftazidime										491	38	49	273		
										57.7	62.2	67.9	100		
Ceftriaxone			203	123	76	39	18	27	22	33	53	57	63	137	
			23.9	38.3	47.2	51.8	53.9	57.1	59.7	63.6	69.8	76.5	83.9	100	
Imipenem				2	337	318	73	18	13	35	19	36			
				0.2	39.8	77.2	85.8	87.9	89.4	93.5	95.8	100			
Levofloxacin	4	42	37	18	31	36	19	33	28	191	412				
	0.5	5.4	9.8	11.9	15.5	19.7	22	25.9	29.1	51.6	100				
Minocycline						44	87	118	130	85	216	171			
						5.2	15.4	29.3	44.5	54.5	79.9	100			
PipTazo					2	22	148	138	117	89	55	38	49	39	154
					0.2	2.8	20.2	36.4	50.2	60.6	67.1	71.6	77.3	81.9	100

*Multi-drug resistance is defined as any bacterial strain that is resistant to 2 or more drug classes of antimicrobial agents.

Table 3. In Vitro Activity (mcg/mL) of Tigecycline and Comparators against *Enterobacteriaceae* Cross-Resistant to Multiple Drug Classes.

Drug	(mcg/mL)	Resistant to 0 Drug Classes (n=1167)	Resistant to 1 Drug Class (n=3742)	Resistant to 2 Drug Classes (n=668)	Resistant to 3 Drug Classes (n=178)	Resistant to 4 Drug Classes (n=5)
Tigecycline	MIC_{50}	0.12	0.5	0.5	1	2
	MIC_{90}	0.5	1	4	4	8
Amikacin	MIC_{50}	2	2	2	2	16
	MIC_{90}	4	4	8	32	32
AmoxClav	MIC_{50}	2	32	16	>32	>32
	MIC_{90}	4	>32	>32	>32	>32
Ampicillin	MIC_{50}	2	>32	>32	>32	>32
	MIC_{90}	16	>32	>32	>32	>32
Cefepime	MIC_{50}	$\leq$0.5	$\leq$0.5	$\leq$0.5	4	>32
	MIC_{90}	$\leq$0.5	1	16	>32	>32
Ceftazidime	MIC_{50}	$\leq$8	$\leq$8	$\leq$8	>32	>32
	MIC_{90}	<8	<8	>32	>32	>32
Ceftriaxone	MIC_{50}	$\leq$0.06	0.12	0.25	32	>64
	MIC_{90}	$\leq$0.06	4	>64	>64	>64
Imipenem	MIC_{50}	0.25	0.5	0.5	0.5	>16
	MIC_{90}	0.5	1	>2	16	>16
Levofloxacin	MIC_{50}	0.03	0.06	8	>8	>8
	MIC_{90}	0.06	0.5	>8	>8	>8
Minocycline	MIC_{50}	1	2	4	16	>16
	MIC_{90}	2	4	>16	>16	>16
PipTazo	MIC_{50}	1	2	4	>32	>128
	MIC_{90}	2	8	>128	>128	>128

CONCLUSIONS

- 89% of all *Enterobacteriaceae* study strains from the United States were resistant to at least one drug class and 15% were cross-resistant to 2 or more drug classes.
- Tigecycline had the lowest in vitro MIC_{90} of all study drugs against MDR strains of *Enterobacteriaceae* and at 4 mcg/mL. This value was 2- to 16-fold lower than imipenem, levofloxacin and cefepime.
- Tigecycline inhibited 82.4% of all multi-drug resistant (resistant to 2 or more drug classes) *Enterobacteriaceae* strains at a MIC value of 2 mcg/mL.
- The MIC_{50} and MIC_{90} values of all study drugs increased as the number of drugs resistant classes against *Enterobacteriaceae* species increased. Tigecycline MICs remained below its susceptible breakpoint value of 2 mcg/mL although resistance to one drug class increased the tigecycline MIC_{90} by 2-fold, and resistance to $\geq$2 classes increased it by 8- to 16-fold.
- The in vitro activity or tigecycline was superior to imipenem, cefepime, ceftazidime, ceftriaxone, levofloxacin, minocycline and piperacillin-tazobactam against all *Enterobacteriaceae* resistant to 2 or more drug classes.
- Existing multi-drug resistant efflux pumps may also pump tigecycline but at a much lower rate. Tigecycline remained effective although resistance to one or two drug classes increased the tigecycline MIC_{90} by 2-fold and resistance to 2 or more drug classes increased the MIC_{90} by as much as 16-fold.