

# Evaluating Multi-Drug Resistant Acinetobacter in a Worldwide Study

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## REVISED ABSTRACT

Background: Tigecycline is a glycylycylone, a new generation of tetracyclines significantly different to be classified as a separate antimicrobial class. Glycylycylones are being developed to overcome the problem of bacterial resistance to tetracyclines and other antimicrobials. Tigecycline is better tolerated and is more active than tetracyclines against a wide variety of Gram-positive and Gram-negative bacteria including *Acinetobacter spp.* The T.E.S.T. program determined the in vitro activity of TIG against *Acinetobacter* resistant to one or more of piperacillin-tazobactam (PT), levofloxacin (LVX), ceftriaxone (CAX), cefepime (CPE), amikacin (AK), minocycline (MIN), ceftazidime (CAZ) and imipenem (IMP). Study strains were collected from 272 hospitals globally throughout 2004-2006.

Methods: A total of 3,334 clinical isolates of *Acinetobacter spp.* were identified to the species level from participating sites 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, with a proposed tigecycline susceptible breakpoint of  $\leq 2$  mcg/ml.

Results: :930/3334 (27.9%) of *Acinetobacter* isolates collected were resistant to 3 or more CLSI drug classes (multi-drug resistant, MDR) TIG inhibited 98.2% of all *Acinetobacter* and 96.2% of MDR *Acinetobacter*. Resistance rates for comparator drugs against MDR *Acinetobacter* were CPE 3%, CAX 0.4%, CAZ 2%, LVX 2%, PT 4%, AK 43%, IMP 54%, and MIN 77%. The modal TIG MIC for strains resistant to 3 or more drug classes was 1 mcg/ml compared to 0.12 mcg/ml strains with no resistant parameters, indicating an 8-fold diminishment of activity.

Conclusion: It has been seen in some species that existing multi-drug efflux pumps may also pump TIG. In spite of this, TIG remained effective and inhibited most *Acinetobacter* strains resistant to 3 or more other drugs in this study, although the higher TIG MICs seen for these strains suggests some linkage to resistance mechanisms for other drugs. TIG remained effective in inhibiting multi-drug resistant *Acinetobacter spp.*, further broadening its wide spectrum of activity vs. drug-resistant bacteria.

## INTRODUCTION

Tigecycline is a novel antimicrobial with expanded broad-spectrum activity from a new class of compounds, the glycylycylones. Tigecycline inhibits protein synthesis by binding to the 30S ribosomal subunit. Although it is perceived to be bacteriostatic, its anti-bacterial activity is significant and has shown some bactericidal activity against key targeted pathogens [1,2]. Tigecycline was developed to provide activity against tetracycline and multi-drug-resistant gram-positive pathogens and has demonstrated significant broad-spectrum activity against aerobic and anaerobic gram-positive and gram-negative microorganisms [2-4].

Tigecycline resistance is very infrequent and is also difficult to induce in the laboratory [5, 6] with a selection frequency observed at less than  $10^{-9}$  [3, 5, 7]. With the exception of *P. aeruginosa*, tetracycline-resistant bacteria with either tetracycline efflux pumps or ribosomal protective features are sensitive to tigecycline [2-4, 7-11]. Tigecycline has shown to be a highly effective against multi-resistant *Acinetobacter spp.*, particularly *A. baumannii* that are commonly associated with serious nosocomial infections. Similar activity has been observed against *Enterobacteriaceae*, even extended-spectrum beta-lactamase (ESBL) and AmpC producing strains [10]. Tigecycline has demonstrated MIC<sub>90</sub> values of  $<0.5$  mcg/ml against methicillin-resistant *Staphylococcus aureus* (MRSA) and other gram-positive organisms [2, 4-6]. Tigecycline has shown potent activity against animal models infected with selected strains of multi-drug resistant *Enterococcus faecium* and *Enterococcus faecalis* [4, 5] with diverse genotypes van-A, -B and -C [6].

This study was designed to better define the in vitro activity of tigecycline against multi-drug resistant *Acinetobacter* clinical isolates in a multi-center, multi-national surveillance.

## MATERIALS & METHODS

- All isolates were derived from blood, respiratory tract, urine (no more than 25% of all isolates), skin, wound, fluids and few other defined sources. Only one isolate per patient was accepted. Isolates were identified to genus and species by the local laboratory. Each site tested the isolates using broth microdilution.
- Clinical isolates (n=3,334) of *Acinetobacter* were collected tested between January 2004 - December 2006 from 272 study centers in 34 countries.
- Custom broth microdilution panels were supplied by MicroScan (Dade MicroScan, Sacramento, CA, USA) with the following antimicrobial agents and concentrations (expressed in mcg/ml): amikacin (0.5-64); 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 for all drugs except tigecycline followed published guidelines established by the CLSI where applicable [12], where available. A proposed susceptibility breakpoint of  $\leq 2$  mcg/mL was defined for tigecycline and *Acinetobacter* in this study for comparative purposes only [13].
- Quality control of broth microdilution panels followed manufacture's and NCCLS guidelines using the following ATCC strains: *Escherichia coli* ATCC 25922; *Escherichia coli* ATCC 35218; and *Pseudomonas aeruginosa* ATCC 27853.
- The collection and transportation of organisms and the confirmation of identification, as well as, construction and management of a centralized database were conducted and coordinated by Laboratories International for Microbiology Studies (LIMS), a subsidiary of International Health Management Associates, Inc. (IHMA, Schaumburg, IL, USA).

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## ACKNOWLEDGEMENTS

This study was supported by a grant from Wyeth Pharmaceuticals. We gratefully acknowledge contributions from the current participants in the T.E.S.T. program who have helped make this program a success.

## RESULTS

The results are listed in the following tables.

Table 1. In vitro (MIC<sub>90</sub>, mcg/mL) activity of tigecycline and comparators for each multi-drug resistant group\*.

Organism	Drug	%Sus <sup>*</sup>	MIC (mcg/mL)		
			MIC <sub>50</sub>	MIC <sub>90</sub>	Range
<i>Acinetobacter spp.</i> (combines all MDR species) (n=930)	Tigecycline	96.2	1	2	$\leq 0.008$ - 8
	Amikacin	42.8	32	>64	$\leq 0.5$ - >64
	Cefepime	3.1	32	>32	$\leq 0.5$ - >32
	Ceftazidime	2.3	>32	>32	$\leq 8$ - >32
	Ceftriaxone	0.4	>64	>64	$\leq 0.06$ - >64
	Imipenem	54.2	4	>16	0.25 - >16
	Levofloxacin	1.6	>8	>8	0.25 - >8
	Minocycline	77.4	1	8	$\leq 0.5$ - >16
PipTazo	4	>128	>128	$\leq 0.06$ - >128	
<i>A. baumannii</i> (n=914)	Tigecycline	96.3	1	2	$\leq 0.008$ - 8
	Amikacin	42.2	32	>64	1 - >64
	Cefepime	3.2	32	>32	$\leq 0.5$ - >32
	Ceftazidime	2.1	>32	>32	$\leq 8$ - >32
	Ceftriaxone	0.4	>64	>64	$\leq 0.06$ - >64
	Imipenem	54.1	4	>16	0.25 - >16
	Levofloxacin	1.5	>8	>8	0.25 - >8
	Minocycline	77.5	1	8	$\leq 0.5$ - >16
PipTazo	3.9	>128	>128	$\leq 0.06$ - >128	
<i>A. anitratus</i> (n=7)	Tigecycline	85.7	--	--	0.25 - 4
	Amikacin	71.4	--	--	4 - >64
	Cefepime	0	--	--	16 - >32
	Ceftazidime	14.3	--	--	$\leq 8$ - >32
	Ceftriaxone	0	--	--	>64 - >64
	Imipenem	42.9	--	--	1 - >16
	Levofloxacin	0	--	--	4 - >8
	Minocycline	57.1	--	--	$\leq 0.5$ - 16
PipTazo	0	--	--	128 - >128	
<i>A. calcoaceticus</i> (n=3)	Tigecycline	100	--	--	1 - 2
	Amikacin	66.7	--	--	2 - 64
	Cefepime	0	--	--	16 - >32
	Ceftazidime	0	--	--	16 - >32
	Ceftriaxone	0	--	--	64 - >64
	Imipenem	100	--	--	1 - 1
	Levofloxacin	0	--	--	>8 - >8
	Minocycline	100	--	--	$\leq 0.5$ - 2
PipTazo	33.3	--	--	$\leq 0.06$ - >128	
<i>A. Iwoffii</i> (n=4)	Tigecycline	100	--	--	0.12 - 1
	Amikacin	100	--	--	$\leq 0.5$ - 16
	Cefepime	0	--	--	16 - >32
	Ceftazidime	25	--	--	$\leq 8$ - >32
	Ceftriaxone	0	--	--	>64 - >64
	Imipenem	25	--	--	2 - >16
	Levofloxacin	25	--	--	0.25 - >8
	Minocycline	100	--	--	$\leq 0.5$ - 2
PipTazo	0	--	--	64 - >128	
<i>Acinetobacter, non-specified</i> (n=2)	Tigecycline	100	--	--	0.25 - 0.5
	Amikacin	50	--	--	1 - >64
	Cefepime	0	--	--	>32 - >32
	Ceftazidime	0	--	--	>32 - >32
	Ceftriaxone	0	--	--	>64 - >64
	Imipenem	100	--	--	0.5 - 4
	Levofloxacin	0	--	--	8 - 8
	Minocycline	50	--	--	1 - 8
PipTazo	0	--	--	>128 - >128	

\* Susceptibilities are defined in CLSI document M100-S16 (2006) where applicable. Tigecycline is not yet approved for treatment of *Acinetobacter*; tigecycline breakpoints are defined as susceptible  $\leq 2$  mcg/mL for comparative purposes only [13].

<sup>1</sup> MDR is defined as resistant to 3 or more drug CLSI classes according to document M100-S16, 2006.

-- MIC<sub>50</sub> and MIC<sub>90</sub> are not calculated for species with n<10.

Table 2. In vitro activity (mcg/mL and % susceptible) of tigecycline and comparative agents against 3,334 *Acinetobacter* clinical isolates worldwide.

Organism	Drug	%Sus <sup>*</sup>	MIC (mcg/mL)		
			MIC <sub>50</sub>	MIC <sub>90</sub>	Range
<i>Acinetobacter spp.</i> (n=3,334)	Tigecycline	98.2	0.25	1	$\leq 0.008$ - 8
	Amikacin	78.6	4	64	$\leq 0.5$ - >64
	Cefepime	51.6	8	>32	$\leq 0.5$ - >32
	Ceftazidime	50.5	$\leq 8$	>32	$\leq 8$ - >32
	Ceftriaxone	33.6	32	>64	$\leq 0.06$ - >64
	Imipenem	86.2	0.5	16	$\leq 0.06$ - >16
	Levofloxacin	52.8	2	>8	$\leq 0.008$ - >8
	Minocycline	90.8	$\leq 0.5$	4	$\leq 0.5$ - >16
PipTazo	59.4	8	>128	$\leq 0.06$ - >128	

\* Susceptibilities are defined in CLSI document M100-S16 (2006) where applicable. Tigecycline is not yet approved for treatment of *Acinetobacter*; tigecycline breakpoints are defined as susceptible  $\leq 2$  mcg/mL for comparative purposes only [13].

Table 3. In vitro activity (mcg/mL and % susceptible) of tigecycline and comparative agents against 990 multi-drug resistant (MDR1) *Acinetobacter* clinical isolates.

Drug	0	MDR Groups / MIC <sub>90</sub> (mcg/mL)					
		1 Drug Class	2 Drug Classes	3 Drug Classes	4 Drug Classes	5 Drug Classes	6 Drug Classes
Tigecycline	0.5	1	2	2	2	2	2
Amikacin	4	32	64	64	64	64	64
Cefepime	8	32	32	32	32	32	32
Ceftazidime	$\leq 8$	32	32	32	32	32	32
Ceftriaxone	16	64	64	64	64	64	64
Imipenem	0.5	2	4	16	16	16	16
Levofloxacin	0.5	8	8	8	8	8	8
Minocycline	$\leq 0.5$	4	8	8	8	8	16
PipTazo	8	64	128	128	128	128	128

\* Multi-drug resistant Group and susceptibilities are defined by CLSI drug class in document M100-S16, 2006. Tigecycline is not yet approved for treatment of *Acinetobacter*; tigecycline breakpoint is defined as susceptible  $\leq 2$  mcg/mL for comparative purposes only [13]. Color coding: Susceptible=blue; Intermediate=orange; Resistant=orange.

## CONCLUSIONS

- Multi-drug resistant (MDR) *Acinetobacter* (resistant to 3 or more CLSI drug classes) comprise more than quarter (28%) of all *Acinetobacter* species collected in this study. 43% were resistant to 2 or more drug classes while over half of all *Acinetobacter* were resistant to at least one antimicrobial agent.
- Tigecycline inhibited 98.2% of all *Acinetobacter* and 96.2% of all MDR *Acinetobacter* in vitro at 2 mcg/mL. Tigecycline MIC<sub>90</sub> values were 4- to >128-fold lower than any other study drug against all *Acinetobacter* and 4- to >64 lower against MDR *Acinetobacter*.
- Tigecycline demonstrated a 4- increase in MIC<sub>90</sub> values between *Acinetobacter* susceptible to all drugs and resistant to two drug classes but no additional increases for resistance to 3 or more drug classes. All other study drugs, with the exception of tigecycline and minocycline, demonstrated MIC<sub>90</sub> values in resistant ranges against *Acinetobacter* isolates resistant to 3 or more drug classes.
- It has been seen in some species that existing multi-drug efflux pumps may also pump tigecycline. In spite of this, TIG remained effective and inhibited most *Acinetobacter* strains resistant to 3 or more other drugs in this study, although the higher tigecycline MICs seen for these strains suggests some linkage to resistance mechanisms for other drugs.