

# Evaluation of Multidrug Resistant (MDR) Isolates Against Tigecycline: A Canadian Perspective

# 66.039

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## REVISED ABSTRACT

**Objectives:** Tigecycline (TIG) is a new glycolcycline with enhanced activity against many multidrug resistant (MDR) pathogens including ESBL and AmpC producing *Enterobacteriaceae*, methicillin-resistant *S. aureus* (MRSA), carbapenem resistant *Acinetobacter* and fluoroquinolone resistant gram-negative rods. The TEST study evaluated the activity of TIG and comparators to pathogens in Canada 2004-2008. **Methods:** A total of 2227 pathogens were collected from 10 participating sites in Canada from 2004-2008. Isolates were identified to the species level and CLSI specified MICs were performed at each site. CLSI or FDA breakpoints were used, where applicable, to determine % susceptibility. **Results:** Tigecycline MICs are recorded in the following table:

Organisms (n=2227)	Tigecycline MICs (mcg/ml)		
	MIC <sub>50</sub>	MIC <sub>90</sub>	Range
<i>Acinetobacter</i> spp. (n=160)	0.12	1	0.015 - 2
<i>Enterobacter</i> spp. (n=300)	0.5	1	0.12 - 8
<i>E. coli</i> (n=334)	0.12	0.25	<0.008 - 2
<i>Klebsiella</i> spp. (n=318)	0.5	2	0.12 - 16
ESBLs (n=13)	1	2	0.25 - 2
<i>Enterococcus</i> spp. (n=181)	0.06	0.12	0.03 - 0.25
VREs (n=7)	0.03	0.06	0.03 - 0.06
<i>Serratia</i> spp. (n=13)	1	2	0.25 - 8
<i>H. influenzae</i> (n=181)	0.25	0.5	0.03 - 2
<i>S. aureus</i> (n=314)	0.12	0.12	0.06 - 0.5
MRSA (n=39)	0.12	0.25	0.06 - 0.5
<i>S. agalactiae</i> (n=12)	0.03	0.06	<0.008 - 0.12
<i>S. pneumoniae</i> (n=188)	0.03	0.03	<0.008 - 0.12

**Conclusions:** Tigecycline showed excellent in vitro activity against a diverse collection of pathogens isolated in Canada between 2004-2008. MIC<sub>50</sub> values of <0.5mcg/ml against most *Enterobacteriaceae* including ESBL and MIC<sub>90</sub> of <0.12mcg/ml against most gram-positive pathogens document the in vitro potency of tigecycline, a new glycolcycline.

## BACKGROUND

Tigecycline is a novel antimicrobial with expanded broad-spectrum activity from a new class of compounds, the glycolcyclines. 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<sup>-9</sup> [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 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>50</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 in selected clinical isolates collected from 10 study centers in Canada and is part of the global Tigecycline Evaluation Susceptibility Study (TEST).

## 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=2227) were collected tested between January 2004 - April 2008 from ten study centers across Canada
- Custom broth microdilution panels were supplied by MicroScan (Dade MicroScan, Sacramento, CA, USA) with the following antimicrobial agents and concentrations (expressed in mcg/ml): Gram Positive Panel: amoxicillin/clavulanic acid (0.03/0.015-8/4, tested using a 2:1 ratio of amoxicillin:clavulanic acid; reported concentrations refer to amoxicillin); ampicillin (0.06-16); ceftriaxone (0.03-64); imipenem (0.06-16); linezolid (0.5-8); levofloxacin (0.06-32); minocycline (0.25-8); tigecycline (0.008-16); piperacillin/tazobactam (0.25/4-16/4) and vancomycin (0.12-32). Gram negative panel: 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 CLSI where applicable [12].
- MIC interpretive criteria for tigecycline followed published guidelines established by the FDA where applicable [13].
- Isolates were identified to genus and species by the local laboratory. Each site tested the isolates using broth microdilution.
- Quality control of broth microdilution panels followed manufacturer's and CLSI guidelines using the following ATCC strains: *Enterococcus faecalis* ATCC 29212; *Escherichia coli* ATCC 25922; *Haemophilus influenzae* ATCC 49247; *Haemophilus influenzae* ATCC 49766; *Staphylococcus aureus* ATCC 29213; *Streptococcus pneumoniae* ATCC 49619; and *Pseudomonas aeruginosa* ATCC 27853.
- ESBL determinations: *E. coli*, *K. pneumoniae* and *K. oxytoca* were screened and confirmed for ESBL activity according to CLSI guidelines. Preliminary ESBL activity was determined by screening ceftriaxone with MICs >1 mcg/mL using broth microdilution panels. ESBL presence was confirmed by testing the following antibiotic disks: cefotaxime (30-mcg), cefotaxime/clavulanic acid (30/10-mcg) and ceftazidime (30-g), ceftazidime/clavulanic acid (30/10-mcg). Antimicrobial disks were manufactured by Oxoid, Inc. (Ogdenburg, NY, USA). Mueller-Hinton agar used in testing was manufactured by Remel, Inc. (Lenexa, KS, USA). An organism was interpreted as containing an ESBL if there was an increase of >5mm in the inhibition zone of the combination disk when compared to that of the cephalosporin alone. Quality control of antimicrobial disks followed manufacturer's guidelines (Oxoid) using the following ATCC strains: *K. pneumoniae* ATCC 700603 (positive ESBL control) and *E. coli* ATCC 25922.
- 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 patients in the T.E.S.T. program who have helped make this program a success.

## RESULTS

Results are contained in the following Tables.

Table 1. In vitro activity of Tigecycline and comparative agents against *Enterobacteriaceae*.

Organism	Drug	%SUS	%INT	%RES	MIC (mcg/ml)		
					MIC <sub>50</sub>	MIC <sub>90</sub>	
All <i>Enterobacteriaceae</i> (n=183)	Tigecycline	96.9	2.2	0.9	0.5	1	
	Amikacin	99.9	0	0.1	2	4	
	Amox/Clav	50.2	7.5	42.3	8	>32	
	Ampicillin	15.1	8.6	76.3	>32	>32	
	Cefepime	97.2	0.6	2.2	<0.5	1	
	Ceftazidime	93	1.9	5.1	<8	<8	
	Ceftriaxone	91.6	2.4	6	<0.06	4	
	Imipenem	100	0	0	0.12	0.5	
	Levofloxacin	87.6	2.1	10.2	0.06	8	
	Minocycline	87.3	6.2	6.6	2	8	
	PipTazo	94	2.7	3.3	1	8	
	<i>Enterobacter aerogenes</i> (n=56)	Tigecycline	96.4	0	3.6	0.5	2
		Amikacin	100	0	0	2	4
		Amox/Clav	1.8	3.6	94.6	>32	>32
Ampicillin		0	8.9	91.1	>32	>32	
Cefepime		100	0	0	<0.5	<0.5	
Ceftazidime		89.3	3.6	7.1	<8	16	
Ceftriaxone		96.4	3.6	0	0.12	2	
Imipenem		100	0	0	0.25	1	
Levofloxacin		94.6	1.8	3.6	0.06	1	
Minocycline		87.5	10.7	1.8	2	8	
PipTazo		94.6	5.4	0	2	16	
<i>Enterobacter cloacae</i> (n=238)		Tigecycline	95.4	3.8	0.8	0.5	1
		Amikacin	99.2	0.3	0.6	2	4
		Amox/Clav	1.6	1	97.4	>32	>32
	Ampicillin	0	5.6	94.4	>32	>32	
	Cefepime	94.9	2	3.1	<0.5	4	
	Ceftazidime	70.8	5.6	23.6	<8	>32	
	Ceftriaxone	74.6	10.4	15	0.25	64	
	Imipenem	100	0	0	0.5	1	
	Levofloxacin	90.5	2.9	6.7	0.03	2	
	Minocycline	87	5.5	7.6	2	8	
	PipTazo	87	5.9	7.1	2	64	
	<i>Escherichia coli</i> (n=334)	Tigecycline	100	0	0	0.12	0.25
		Amikacin	100	0	0	4	16
		Amox/Clav	35	55	10	16	16
Ampicillin		0	0	100	>32	>32	
Cefepime		30	5	65	32	>32	
Ceftazidime		50	15	35	16	>32	
Ceftriaxone		5	10	85	>64	>64	
Imipenem		100	0	0	0.25	0.25	
Levofloxacin		10	5	85	>8	>8	
Minocycline		65	15	20	2	16	
PipTazo		95	5	0	2	16	
<i>Klebsiella pneumoniae</i> (n=259)		Tigecycline	93.8	5	1.2	0.5	2
		Amikacin	100	0	0	1	2
		Amox/Clav	91.1	5.8	3.1	2	8
	Ampicillin	0.8	22.4	76.8	32	>32	
	Cefepime	98.1	0.4	1.5	<0.5	<0.5	
	Ceftazidime	95	1.9	3.1	<8	<8	
	Ceftriaxone	95	1.9	3.1	<0.06	0.25	
	Imipenem	100	0	0	<0.06	0.5	
	Levofloxacin	93.1	1.5	5.4	0.06	1	
	Minocycline	83	6.6	10.4	2	16	
	PipTazo	96.9	1.2	1.9	2	8	
	<i>Klebsiella oxytoca</i> (n=57)	Tigecycline	98.2	0	1.8	0.25	0.5
		Amikacin	100	0	0	2	2
		Amox/Clav	84.2	5.3	10.5	2	32
Ampicillin		0	8.8	91.2	>32	>32	
Cefepime		100	0	0	<0.5	1	
Ceftazidime		100	0	0	<8	<8	
Ceftriaxone		98.2	0	1.8	<0.06	2	
Imipenem		100	0	0	<0.06	0.5	
Levofloxacin		96.5	3.5	0	0.03	0.25	
Minocycline		96.5	3.5	0	1	2	
PipTazo		87.7	0	12.3	1	>128	
<i>S. marcescens</i> (n=127)		Tigecycline	97.6	1.6	0.8	1	2
		Amikacin	100	0	0	1	1
		Amox/Clav	0	100	0	16	16
	Ampicillin	0	0	100	32	32	
	Cefepime	100	0	0	4	4	
	Ceftazidime	100	0	0	<8	<8	
	Ceftriaxone	100	0	0	2	2	
	Imipenem	100	0	0	0.5	0.5	
	Levofloxacin	0	0	100	>8	>8	
	Minocycline	0	0	100	>16	>16	
	PipTazo	100	0	0	4	4	
	All ESBL producers <i>E. coli</i> , <i>K. pneumoniae</i> , <i>K. oxytoca</i> (n=33)	Tigecycline	100	0	0	0.25	1
		Amikacin	100	0	0	4	8
		Amox/Clav	42.4	48.5	9.1	16	16
Ampicillin		0	0	100	>32	>32	
Cefepime		42.4	6.1	51.5	32	>32	
Ceftazidime		39.4	18.2	42.4	16	>32	
Ceftriaxone		15.2	15.2	69.7	>64	>64	
Imipenem		100	0	0	0.25	0.25	
Levofloxacin		24.2	6.1	69.7	>8	>8	
Minocycline		63.6	15.2	21.2	2	>16	
PipTazo		90.9	6.1	3	4	16	
Levofloxacin Resistant <i>Enterobacteriaceae</i> (n=111)		Tigecycline	94.6	3.6	1.8	0.25	2
		Amikacin	99.1	0	0.9	2	8
		Amox/Clav	48.6	30.6	20.7	16	>32
	Ampicillin	13.5	0	86.5	>32	>32	
	Cefepime	79.3	1.8	18.9	<0.5	>32	
	Ceftazidime	80.2	5.4	14.4	<8	>32	
	Ceftriaxone	69.4	4.5	26.1	0.12	>64	
	Imipenem	100	0	0	0.25	0.5	
	Levofloxacin	0	0	100	>8	>8	
	Minocycline	68.5	13.5	18	2	16	
	PipTazo	89.2	5.4	5.4	2	32	
	AmpC positive <i>Enterobacter</i> spp. & <i>S. marcescens</i> (n=22)	Tigecycline	90.9	9.1	0	0.5	2
		Amikacin	100	0	0	1	4
		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	0.25	0.5	
Levofloxacin		90.9	0	9.1	0.03	2	
Minocycline		81.8	9.1	9.1	4	8	
PipTazo		4.5	36.4	59.1	128	>128	

Table 2. In vitro activity of Tigecycline and comparative agents against *Acinetobacter baumannii* and *Pseudomonas aeruginosa*.

Organism	Drug	%SUS	%INT	%RES	MIC (mcg/mL)		
					MIC <sub>50</sub>	MIC <sub>90</sub>	
<i>Acinetobacter baumannii</i> (n=129)	Tigecycline	na	na	na	0.25	1	
	Amikacin	89.9	2.3	7.8	2	32	
	Cefepime	na	na	na	16	32	
	Ceftazidime	na	na	na	16	>32	
	Ceftriaxone	77.5	10.1	12.4	4	32	
	Imipenem	80.6	10.1	9.3	<8	16	
	Levofloxacin	43.4	41.9	14.7	16	>64	
	Minocycline	98.4	1.6	0	<0.5	<0.5	
	PipTazo	86	8.5	5.4	0.25	64	
	<i>Acinetobacter</i> spp. Carbapenem R (n=6)	Tigecycline	na	na	na	1	2
		Amikacin	66.7	0	33.3	16	>64
		Cefepime	0	0	100	>32	>32
		Ceftazidime	0	0	100	>32	>32
		Ceftriaxone	0	0	100	>64	>64
Imipenem		0	0	100	>16	>16	
Levofloxacin		0	16.7	83.3	8	>8	
Minocycline		100	0	0	<0.5	2	
PipTazo		0	16.7	83.3	>128	>128	
<i>Pseudomonas aeruginosa</i> (n=273)		Tigecycline	na	na	na	8	16
		Amikacin	96	1.1	2.9	4	8
		Cefepime	84.2	11	4.8	4	16
		Ceftazidime	88.6	5.5	5.9	<8	16
		Ceftriaxone	20.9	38.8	40.3	32	>64
	Imipenem	87.8	2.9	9.4	1	8	
	Levofloxacin	72.9	8.4	18.7	0.5	>8	
	Minocycline	9.5	22.7	67.8	16	>16	
	PipTazo	94.5	0	5.5	4	32	