

REVISED ABSTRACT

Background: The TEST program determined the in vitro activity of tigecycline compared to broad spectrum antimicrobials against multidrug resistant gram-negative and gram-positive species collected from hospitals within Turkey throughout 2004-2006. **Methods:** Clinical isolates were identified to the species level and confirmed by a reference laboratory. Minimum Inhibitory Concentration (MICs) were determined by the local laboratory using supplied broth microdilution panels and interpreted according to CLSI guidelines. **Results:** Out of 347 isolates collect, 130 (37%) were determined to be multidrug resistant as defined by resistant to 3 or more antimicrobial drug classes. *A. baumannii* had the highest percentage of MDR strains with 26/30 (87%) followed in order by *Klebsiella spp.* 24/49 (49%); *E. coli* 25/54 (46%); *P. aeruginosa* 18/42 (43%); *Enterobacter spp.* 12/29 (41%); *S. marcescens* 9/23 (39%) and *S. aureus* 13/48 (27%). Of these, 80% or more were nosocomial infections in adults over 30 years age. Tigecycline inhibited 84% of the gram-negative MDR strains at 2 mcg/ml compared to the next most active drug, imipenem (78.6% at 4 mcg/ml). All 16 MDR gram-positive strains (mostly MRSA) were inhibited by linezolid at 4 mcg/ml, vancomycin at 2 mcg/ml and tigecycline at 0.5 mcg/ml. **Conclusion:** Turkey had the highest level of MDR strains (37%) of any country seen to-date in the TEST program. Tigecycline demonstrated significant in vitro activity against more than 80% of all the MDR strains from these sites with activity similar to linezolid and vancomycin, and more potent than imipenem.

INTRODUCTION

Tigecycline (formerly GAR-936) is a member of a new class of antimicrobial agents, the glycyclines. 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 tigecycline and other glycyclines 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 [3].

Previous studies have demonstrated excellent in vitro activity for tigecycline against clinical and laboratory strains of gram-positive and -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* [4-6]. This study was undertaken to document the in vitro activity of tigecycline against significant numbers of clinical pathogens collected from a large geographically diverse population over three years time. This study is part of the ongoing global Tigecycline Evaluation and Surveillance Trials (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. There were 347 clinical isolates were collected and tested from 2004 to 2006 from 2 investigative sites in Turkey. 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.
- 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 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); ampicillin (0.5-32, gram-negative panel, and 0.06-16, gram-positive panel); cefepime (0.5-32); ceftazidime (0.06-64); ceftazidime (8-32); imipenem (0.06-16); linezolid (0.5-8); levofloxacin (0.008-8); minocycline (0.5-16); tigecycline (0.008-16); penicillin (0.06-8); piperacillin/tazobactam (0.06/4-128/4) and vancomycin (0.12-32). 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: *E. coli* ATCC 25922; *E. coli* ATCC 35218; *H. influenzae* ATCC 49766; *H. influenzae* ATCC 49247; *S. aureus* ATCC 29213; *Pseudomonas aeruginosa* ATCC 27853; *Enterococcus faecalis* ATCC 29212 and *S. pneumoniae* ATCC 49619. Results were included in the analysis only when corresponding QC isolates tested within the acceptable range according to CLSI (2006) guidelines [8].

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RESULTS

Table 2. Comparative in vitro activity of tigecycline against 130 multidrug resistant clinical isolates from Turkey.*

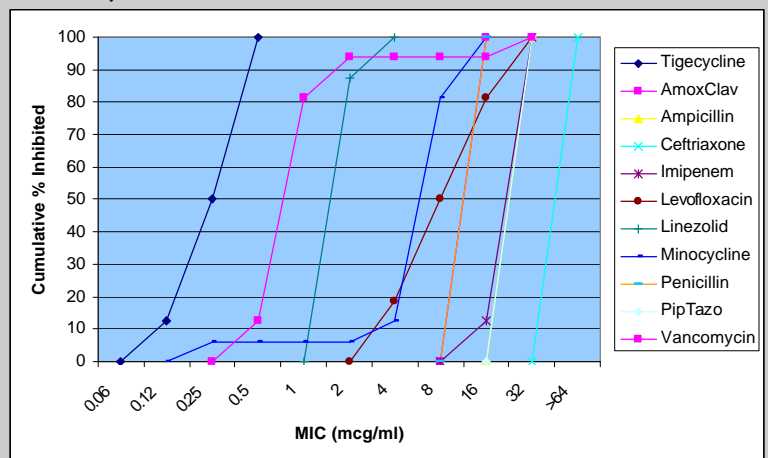
Organisms; MDR (Total N)	Drug	MIC (mcg/ml)			
		MIC50	MIC90	Range	%Sus ^a %Res
Gram-Positives					
<i>Staphylococcus aureus</i> , MRSA n=13 (48)	Tigecycline	0.5	0.5	0.25-0.5	100 0
	AmoxClav	>8	>8	>8->8	0 100
	Ampicillin	>16	>16	>16->16	0 100
	Ceftriaxone	>64	>64	>64->64	0 100
	Imipenem	>16	>16	16->16	0 100
	Levofloxacin	8	16	4-16	0 100
	Linezolid	2	4	2-4	100 0
	Minocycline	8	8	4->8	7.7 7.7
	Penicillin	>8	>8	>8->8	0 100
	PipTazo	>16	>16	>16->16	0 100
Vancomycin	1	1	0.5-2	100 0	
<i>Enterococcus faecium</i> n=3 (28)	Tigecycline	0.12	0.25	0.12-0.25	100 0
	Ampicillin	>16	>16	>16->16	0 100
	Levofloxacin	>32	>32	>32->32	0 100
	Linezolid	2	2	38750	100 0
	Minocycline	>8	>8	≤0.25->8	33.3 66.7
	Penicillin	>8	>8	>8->8	0 100
	Vancomycin	2	>32	0.5->32	66.7 33.3
Gram-Negatives					
<i>Acinetobacter baumannii</i> n=26 (30)	Tigecycline b	1	2	0.5-4	96.2 0
	Amikacin	>64	>64	2->64	38.5 57.7
	Cefepime	32	>32	16->32	0 96.2
	Ceftazidime	>32	>32	>32->32	0 100
	Ceftriaxone	>64	>64	64->64	0 100
	Imipenem	2	>16	0.5->16	53.8 46.2
	Levofloxacin	8	>8	8->8	0 100
	Minocycline	1	8	≤0.5-16	88.5 3.8
	PipTazo	>128	>128	64->128	0 96.2
	<i>Enterobacter spp.</i> ^c n=12 (29)	Tigecycline	0.5	4	0.5-8
Amikacin		2	16	1-32	91.7 0
AmoxClav		>32	>32	>32->32	0 100
Ampicillin		>32	>32	>32->32	0 100
Cefepime		8	8	2-16	91.7 0
Ceftazidime		>32	>32	32->32	0 100
Ceftriaxone		64	>64	2->64	8.3 58.3
Imipenem		0.5	1	0.25-2	100 0
Levofloxacin		0.12	>8	0.03->8	66.7 16.7
Minocycline		2	16	2->16	66.7 16.7
<i>Escherichia coli</i> n=25 (54)	Tigecycline	0.5	1	0.25-2	100 0
	Amikacin	4	16	1-64	92 0
	AmoxClav	32	>32	16->32	0 96
	Ampicillin	>32	>32	32->32	0 100
	Cefepime	>32	>32	≤0.5->32	12 84
	Ceftazidime	16	>32	≤8->32	28 48
	Ceftriaxone	>64	>64	1->64	4 88
	Imipenem	0.5	0.5	0.25-2	100 0
	Levofloxacin	>8	>8	0.03->8	4 96
	Minocycline	8	>16	2->16	32 44
<i>Klebsiella spp.</i> ^d n=24 (49)	Tigecycline	1	2	0.5-2	100 0
	Amikacin	4	32	1-64	79.2 4.2
	AmoxClav	32	>32	16->32	0 95.8
	Ampicillin	>32	>32	>32->32	0 100
	Cefepime	>32	>32	≤0.5->32	37.5 62.5
	Ceftazidime	32	>32	≤8->32	12.5 75
	Ceftriaxone	>64	>64	0.12->64	16.7 70.8
	Imipenem	0.5	1	0.25-1	100 0
	Levofloxacin	0.12	>8	0.03->8	83.3 16.7
	Minocycline	4	>16	2->16	62.5 29.2
<i>Pseudomonas aeruginosa</i> n=18 (42)	Tigecycline b	>16	>16	0.5->16	6.3 93.8
	Amikacin	16	32	2-32	62.5 0
	Cefepime	>32	>32	4->32	12.5 75
	Ceftazidime	78	>32	≤8->32	50 43.8
	Ceftriaxone	>64	>64	64->64	0 100
	Imipenem	16	16	0.25-16	25 56.3
	Levofloxacin	>8	>8	1->8	12.5 81.3
	Minocycline	>16	>16	16->16	0 100

Table 1. Multidrug resistance (MDR) per country in the T.E.S.T. program ranked by % MDR*

Country	MDR (Total N)	% MDR	Country	MDR (Total N)	% MDR
Turkey	130 (347)	37.5	Latvia	50 (378)	13.2
Chile	136 (370)	36.8	United Kingdom	98 (753)	13
Mexico	70 (190)	36.8	Austria	23 (185)	12.4
Greece	170 (518)	32.8	United States	3,746 (30,096)	12.4
Brazil	60 (187)	32.1	France	200 (1,646)	12.2
Korea	110 (354)	31.1	Philippines	36 (297)	12.1
Poland	55 (181)	30.4	Australia	54 (500)	10.8
Pakistan	90 (299)	30.1	Spain	161 (1,485)	10.8
China	103 (345)	30	Germany	141 (1,504)	9.4
Argentina	460 (1,697)	27.1	Ireland	35 (378)	9.3
India	62 (229)	27.1	Switzerland	28 (375)	7.5
Italy	471 (1,940)	24.3	Canada	56 (773)	7.2
Czech Republic	24 (141)	17	Denmark	5 (169)	3
Portugal	32 (194)	16.5	The Netherlands	4 (196)	2
Hungary	35 (228)	15.4	Finland	3 (155)	1.9
Belgium	109 (714)	15.3	Sweden	3 (354)	0.8
South Africa	101 (682)	14.8			
Singapore	54 (372)	14.5	Total (34 countries)	6,915 (48,232)	14.3

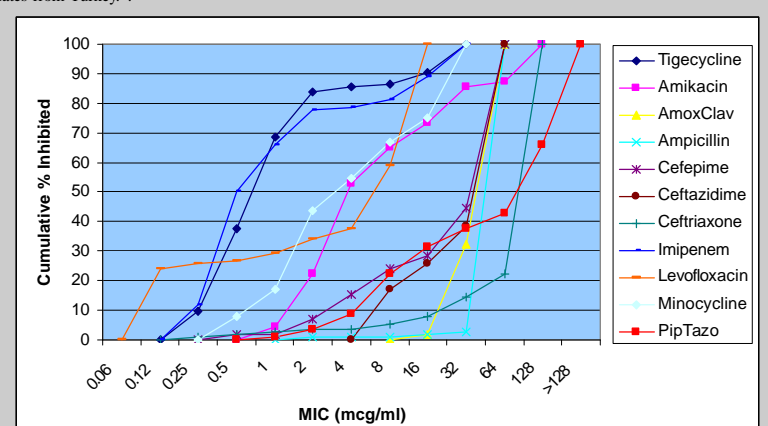
* Data collected from 271 investigative sites.

Figure 1. Cumulative percents inhibited by tigecycline and comparators against 16 multidrug resistant gram-positive isolates from Turkey.*



* *S. aureus*, MRSA (13); *E. faecium* (3)

Figure 2. Cumulative percents inhibited by tigecycline and comparators against 114 multidrug resistant gram-negative isolates from Turkey.*



* *A. baumannii* (26); *E. coli* (25); *E. aerogenes* (2); *E. agglomerans* (1); *E. cloacae* (9); *K. oxytoca* (2); *K. pneumoniae* (22); *P. aeruginosa* (18); *S. marcescens* (9);

CONCLUSIONS

- There were 9 of 34 countries that demonstrated MDR isolate rates equal to or greater than 30% in the TEST program. The highest level of MDR isolates was seen in Turkey (37.5%) and the lowest seen in Sweden (0.8%).
- All 16 gram-positive MDR isolates from Turkey were inhibited by tigecycline, linezolid, and vancomycin at their respective breakpoints.
- Tigecycline was the most potent drug in vitro against MDR *Acinetobacter baumannii*, while imipenem was the most potent antimicrobial agent against MDR *Enterobacter*. Tigecycline and imipenem inhibited 100% of all MDR *E. coli*, *Klebsiella spp.*, and *S. marcescens* at their respective breakpoints.
- None of the study drugs, including piperacillin-tazobactam, were very active against MDR *P. aeruginosa*. Amikacin was the most active drug against these isolates, inhibiting 62.5% at its susceptible breakpoint of <16 mcg/ml.
- During the 3 years covered by this analysis from Turkey, tigecycline has fully retained excellent activity against a broad spectrum of bacteria, including many strains resistant to multiple antimicrobials.