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Activity of Tigecycline Tested Against Non-Fermentative Gram-Negative Bacilli, Including Imipenem-Non-Susceptible Strains, and ESBL-producing Enterobacteriaceae

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ABSTRACT

Background: Resistance among Gram-negative pathogens to expanded spectrum ß-lactams and carbapenems is a global concern with few new antimicrobials entering into development. Tigecycline (TIG), a novel broad spectrum glycylcycline, is currently under priority review by the FDA for treatment of infections of skin and soft tissues, and intra-abdominal infections. This study compares the activity of TIG against select resistant (R) pathogens (including imipenem-non-susceptible [IMP-NS], non-fermentative bacilli [NFB] and ESBL-producing Enterobacteriaceae [ENT]).

Methods: All strains collected from a TIG global surveillance program (2000-2004) were centrally processed by CLSI reference broth microdilution methods and included *Acinetobacter* spp. (ACB; 593 strains), *Burkholderia cepacia* (BUC; 42), *Stenotrophomonas maltophilia* (STM; 341), *E. coli* (EC; 5,326), and *Klebsiella* spp. (KSP; 2,204). ESBL production was confirmed using the CLSI disk approximation method.

Results: $MIC_{50/90}$ values and cumulative inhibition rates for TIG are in the Table.

Organism (no. tested)	MIC (µg/ml) 50%/90%	Cumulative % inhibited at (µg/ml): 0.5	1	2	4	8
ACB						
IMP-S (484)	0.5/2	56	76	95	99	100
IMP-NS (109)	1/4	15	50	88	99	100
BUC (42)	2/8	21	48	69	86	98
STM (341)	1/2	38	73	91	98	100
EC						
ALL (5,326)	0.12/0.25	>99	>99	>99	100	-
ESBL-confirmed (263)	0.25/0.5	99	>99	100	-	-
KSP						
ALL (2,204)	0.5/1	83	94	98	100	-
ESBL-confirmed (389)	0.5/2	63	84	96	100	-

All ACB, STM and 98% of BUC were inhibited by 8 µg/ml of TIG. $MIC_{50/90}$ values of IMP-NS ACB were only two-fold above those of IMP-S strains and in both instances, 99% of strains were inhibited at 4 µg/ml. All EC and KSP were inhibited by 4 µg/ml TIG, including ESBL-producing subsets, with ESBL $MIC_{50/90}$ values being only two-fold higher than the overall populations.

Conclusions: Remarkable potency (98% at 2 µg/ml, > 99% at 4 µg/ml) of TIG was observed against inherently-R, NFB populations (including IMP-NS strains), and confirmed ESBL-producing EC and KSP. TIG may represent a welcome addition to the few remaining parenteral agents active against these R Gram-negative pathogens.

INTRODUCTION

Dramatic increases in the prevalence of extended spectrum ß-lactamase- (ESBL) producing Enterobacteriaceae (primarily *E. coli* and *Klebsiella* spp.) and multidrug-resistant nonfermentative Gram-negative bacilli are changing the face of empiric antimicrobial therapy in health care settings that deal with a high proportion of seriously ill patients, often with co-morbidities. Resistance to "third"- and "fourth-generation" cephalosporins, ß-lactam/ß-lactamase inhibitor combinations, and even carbapenems has become commonplace in various geographic regions, requiring the utilization of unproven antimicrobic 'cocktails' or 'agents of last resort', such as polymyxin B and colistin. New agents to combat these resistant pathogens are sorely needed.

Tigecycline is the first member of the glycylcycline class to be advanced into clinical trials and was recently (June, 2005) licensed by the United States Food and Drug Administration (USA-FDA) as a parenteral agent for the treatment of complicated skin and skin structure and intra-abdominal infections. Although chemically related to the tetracyclines and displaying identical binding sites on the 30S ribosomal subunit, tigecycline binds to the target much more avidly and offers enhanced stability to the usual tetracycline efflux and ribosomal protection resistance mechanisms.

A variety of recent studies have addressed the enhanced spectrum of tigecycline, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* spp. (VRE), penicillin-resistant *Streptococcus pneumoniae* (PRSP), Enterobacteriaceae, certain non-fermentative bacilli, anaerobic wound pathogens, *Haemophilus influenzae, Moraxella catarrhalis, Neisseria gonorrhoeae*, chlamydiae and mycoplasmas. Only members of the tribe *Proteae* and *Pseudomonas aeruginosa* display predictably elevated tigecycline MIC values. Tigecycline is known to be active against the majority of *Acinetobacter* spp. at clinically achievable concentrations.

In this study we evaluated the in vitro activity of tigecycline against a large collection of Enterobacteriaceae (including ESBL-producing strains), and several of the more commonly occurring non-fermentative Gram-negative pathogens (other than pseudomonads) including *Stenotrophomonas maltophilia, Burkholderia cepacia* and *Acinetobacter* spp. (carbapenem-susceptible and -resistant populations) recovered predominantly from patients with bloodstream, respiratory tract, skin and skin structure and urinary tract infections.

MATERIALS AND METHODS

Specimen Collection. The collection consisted of 8,506 consecutively acquired, non-duplicate, patient isolates submitted from nearly 100 participating medical centers representing over 25 countries in Asia, Australia, Europe, North America, and South America. Isolates were identified by the submitting laboratories and subsequently shipped to the monitor (JMI Laboratories, Iowa, USA) where identifications were confirmed using traditional methods and included use of an automated system (Vitek, bioMerieux, Hazelwood, MO, USA).

Susceptibility Testing Methods. MIC values for tigecycline and comparator agents were tested using validated, dry-form broth microdilution panels (TREK Diagnostics, Cleveland, OH) with fresh cation-adjusted Mueller-Hinton broth medium. Testing, incubation and MIC interpretations were performed using the manufacturers recommendations and suggested technical details of the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS; 2003 and 2005).Tigecycline susceptibility breakpoint interpretations as defined by the USA-FDA for Enterobacteriaceae indicate that ≤ 2 µg/ml is susceptible, 4 µg/ml is intermediate and ≥ 8 µg/ml resistant. These criteria were also applied to the non-fermentative species presented for comparison purposes only.

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RESULTS

- All 8,506 strains presented here from the years 2000-2004 were inhibited by ≤ 8 µg/ml of tigecycline (exception, one strain of *B. cepacia*; MIC, 16 µg/ml); 93.4% of *Acinetobacter* spp, 91.5% of *S. maltophilia*, 69.0% of *B. cepacia*, 99.9% of *E. coli* and 97.9% of *Klebsiella* spp. were inhibited by ≤ 2 µg/ml, the USA-FDA approved breakpoint for Enterobacteriaceae (Tables 1 to 3).

Table 1. Activity of tigecycline and comparator antimicrobial agents tested against 5,326 *E. coli* strains (263 confirmed ESBL-producers) and 2,204 *Klebsiella* spp. (389 confirmed ESBL-producers).

Organism (no. tested)	MIC (µg/ml) 50%	90%	Range	% susceptible/resistant[a]
E. coli (5,326)				
Tigecycline	0.12	0.25	0.03-4	99.9/0.0
Tetracycline	≤2	>8	≤2->8	65.2/34.3
Ampicillin	16	>16	≤1->16	49.1/49.8
Amoxicillin/clavulanic acid	8	16	≤1->16	79.2/5.9
Piperacillin/tazobactam	2	4	≤0.12->256	96.0/1.9
Cefuroxime	4	16	≤0.12->16	89.8/6.6
Ceftriaxone	≤0.25	≤0.25	≤0.25->32	95.6/3.6
Ceftazidime	≤1	≤1	≤1->16	96.2/2.4
Cefepime	≤0.12	≤0.12	≤0.12->16	97.1/2.3
Imipenem	≤0.5	≤0.5	≤0.5->8	99.9/0.0
Ciprofloxacin	≤0.03	>4	≤0.03->4	84.1/15.9
Levofloxacin	≤0.03	>4	≤0.03->4	84.2/13.7
Amikacin	2	4	≤0.25->32	99.4/0.3
Gentamicin	≤2	≤2	≤2->8	92.0/7.3
Trimethoprim/sulfamethoxazole	≤0.05	>2	≤0.5->2	68.8/31.2
E. coli (ESBL-confirmed; 263)				
Tigecycline	0.25	0.5	0.03-2	100.0/0.0
Tetracycline	>8	>8	≤2->8	26.3/72.5
Ampicillin	>16	>16	16->16	0.0/99.2
Amoxicillin/clavulanic acid	16	>16	2->16	20.9/30.0
Piperacillin/tazobactam	8	>64	0.5->64	70.7/14.1
Cefuroxime	>16	>16	0.25->16	4.2/88.2
Ceftriaxone	>32	>32	≤0.25->32	24.0/66.9
Ceftazidime	16	>16	≤1->16	42.2/38.0
Cefepime	16	>16	≤0.12->16	45.6/45.2
Imipenem	≤0.5	≤0.5	≤0.5	100.0/0.0
Ciprofloxacin	>4	>4	≤0.03->4	33.5/66.5
Levofloxacin	>4	>4	≤0.03->4	33.8/60.8
Amikacin	4	32	0.5->32	89.4/4.6
Gentamicin	≤2	>8	≤2->8	51.7/43.7
Trimethoprim/sulfamethoxazole	>2	>2	≤0.5->2	44.1/55.9
Klebsiella spp.[b] (2,204)				
Tigecycline	0.5	1	0.06-8	97.9/0.0
Tetracycline	≤2	>8	≤2->8	79.9/16.7
Ampicillin	>16	>16	≤1->16	4.8/79.6
Amoxicillin/clavulanic acid	2	16	≤1->16	77.7/9.1
Piperacillin/tazobactam	2	>64	≤0.12->64	86.3/10.8
Cefuroxime	2	>16	≤0.12->16	76.3/19.1
Ceftriaxone	≤0.25	>32	≤0.25->32	83.7/11.5
Ceftazidime	≤1	>16	≤1->16	86.1/11.5
Cefepime	≤0.12	8	≤0.12->16	90.4/8.1
Imipenem	≤0.5	≤0.5	≤0.5->8	99.5/0.3
Ciprofloxacin	≤0.03	2	≤0.03->4	88.3/10.0
Levofloxacin	0.06	2	≤0.03->4	90.2/7.9
Amikacin	1	16	≤0.25->32	93.1/4.0
Gentamicin	≤2	>8	≤2->8	84.5/13.9
Trimethoprim/sulfamethoxazole	≤0.5	>2	≤0.5->2	80.6/19.4
Klebsiella spp.[c] (ESBL-confirmed; 389)				
Tigecycline	0.5	2	0.12-4	95.6/0.0
Tetracycline	4	>8	≤2->8	52.7/38.6
Ampicillin	>16	>16	>16	0.0/100.0
Amoxicillin/clavulanic acid	16	>16	4->16	23.9/27.8
Piperacillin/tazobactam	32	256	0.25->256	49.1/39.1
Cefuroxime	>16	>16	0.25->16	6.2/87.1
Ceftriaxone	>32	>32	≤0.25->32	19.5/56.3
Ceftazidime	>16	>16	≤1->16	32.5/55.7
Cefepime	8	>16	≤0.12->16	54.8/38.3
Imipenem	≤0.5	≤0.5	≤0.5-4	100.0/0.0
Ciprofloxacin	0.5	>4	≤0.03->4	58.8/35.1
Levofloxacin	0.5	>4	≤0.03->4	66.1/28.8
Amikacin	8	>32	≤0.25->32	69.4/19.0
Gentamicin	>8	>8	≤2->8	30.8/62.2
Trimethoprim/sulfamethoxazole	>2	>2	≤0.5->2	43.2/56.8

a. Criteria as published by the CLSI [2005] or USA-FDA (tigecycline).
b. Includes: *Klebsiella ornithinolytica* (two strains), *K. oxytoca* (371 strains), *K. ozaenae* (four strains), *K. pneumoniae* (1,795 strains), *Klebsiella* spp. (31 strains) and *K. terrigena* (one strain).
c. Includes: *K. oxytoca* (39 strains), *K. ozaenae* (one strain), *K. pneumoniae* (345 strains), and *Klebsiella* spp. (four strains).

- Among those agents tested against *E. coli*, the rank order of susceptibility was: tigecycline = imipenem (MIC_{90} values 0.25 and ≤ 0.5 µg/ml, respectively; both 99.9% susceptible) > amikacin (99.4%) > cefepime (97.1%) > ceftazidime (96.2%) > ceftriaxone (95.6%). The only agents that displayed > 90% susceptibility to confirmed ESBL-producing *E. coli* were tigecycline and imipenem (MIC_{90} values, 0.5 and ≤ 0.5 µg/ml, respectively; 100% susceptible; Table 1).

Table 2. Activity of tigecycline and comparator antimicrobial agents tested against *Acinetobacter* spp. (484 strains, imipenem-susceptible; 109 strains, imipenem-non-susceptible), *Stenotrophomonas maltophilia* (341 strains) and *Burkholderia cepacia* (42 strains).

Organism (no. tested)	MIC (µg/ml) 50%	90%	Range	% susceptible/resistant[a]
Acinetobacter spp.[b] (484 strains)				
Imipenem-susceptible				
Tigecycline	0.5	2	0.06-8	94.6/0.8
Tetracycline	4	>8	≤2->8	58.1/26.7
Ampicillin/sulbactam	8	>32	≤0.25->32	65.3/23.8
Piperacillin/tazobactam	32	128	≤0.12->256	44.6/41.3
Ticarcillin/clavulanic acid	32	>128	≤16->128	39.5/37.8
Ceftazidime	16	>16	≤1->16	45.0/47.9
Cefepime	8	>16	≤0.12->16	50.8/36.2
Imipenem	≤0.5	2	≤0.5-4	100.0/0.0
Ciprofloxacin	>4	>4	≤0.03->4	46.7/52.5
Levofloxacin	2	>4	≤0.03->4	50.6/44.0
Amikacin	4	>32	≤0.25->32	64.7/32.0
Gentamicin	4	>8	≤2->8	53.5/42.4
Tobramycin	1	>16	≤0.12->16	70.5/25.8
Trimethoprim/sulfamethoxazole	2	>2	≤0.5->2	50.8/49.2
Polymyxin B	≤1	≤1	≤1->8	99.0/0.8
Acinetobacter spp.[c] (109 strains)				
Imipenem-non-susceptible				
Tigecycline	1	4	0.12-8	88.1/0.9
Tetracycline	>8	>8	≤2->8	12.0/67.6
Ampicillin/sulbactam	32	>32	4->32	9.2/71.6
Piperacillin/tazobactam	>64	>64	4->64	0.9/97.2
Ticarcillin/clavulanic acid	>128	>128	64->128	0.0/98.2
Ceftazidime	>16	>16	4->16	5.5/92.7
Cefepime	>16	>16	2->16	8.3/65.1
Imipenem	>8	>8	8->8	0.0/87.2
Ciprofloxacin	>4	>4	0.12->4	2.8/96.3
Levofloxacin	>4	>4	0.06->4	8.3/84.4
Amikacin	>32	>32	0.5->32	13.8/78.0
Gentamicin	>8	>8	≤2->8	9.3/82.4
Tobramycin	>16	>16	0.25->16	17.4/78.0
Trimethoprim/sulfamethoxazole	>2	>2	≤0.5->2	12.8/87.2
Polymyxin B	≤1	≤1	≤1->8	98.2/1.8
Stenotrophomonas maltophilia (341)				
Tigecycline	1	2	0.12-8	91.5/2.6
Tetracycline	>8	>8	≤2->8	3.8/69.3
Piperacillin/tazobactam	>64	>64	2->64	9.7/66.0
Ticarcillin/clavulanic acid	32	128	≤16->128	47.8/17.3
Ceftazidime	8	>16	≤1->16	53.2/35.9
Cefepime	16	>16	0.5->16	17.3/49.0
Imipenem	>8	>8	2->8	1.5/98.2
Ciprofloxacin	2	>4	0.12->4	28.7/38.1
Levofloxacin	1	4	0.12->4	86.2/6.2
Amikacin	>32	>32	0.5->32	8.8/86.5
Gentamicin	>8	>8	≤2->8	8.2/89.1
Tobramycin	>16	>16	0.25->16	6.7/90.0
Trimethoprim/sulfamethoxazole	≤0.5	1	≤0.5->2	97.4/2.6
Polymyxin B	≤1	4	≤1->8	86.1/13.9
Burkholderia cepacia (42)				
Tigecycline	2	8	0.03-16	69.0/14.3
Tetracycline	>8	>8	≤2->8	4.9/95.1
Piperacillin/tazobactam	4	32	0.5->256	83.3/7.1
Ticarcillin/clavulanic acid	>128	>128	≤16->128	9.5/78.6
Ceftazidime	4	8	≤1->16	95.2/4.8
Cefepime	8	>16	≤0.12->16	73.8/11.9
Imipenem	4	8	≤0.5->8	66.7/7.1
Ciprofloxacin	1	4	0.06->4	59.5/21.4
Levofloxacin	2	>4	≤0.03->4	81.0/11.9
Amikacin	>32	>32	0.5->32	11.9/81.0
Gentamicin	>8	>8	≤2->8	7.1/90.5
Tobramycin	>16	>16	≤0.12->16	7.1/88.1
Trimethoprim/sulfamethoxazole	≤0.5	>2	≤0.5->2	88.1/11.9
Polymyxin B	>8	>8	≤1->8	9.8/87.8

a. Criteria as published by the CLSI [2005]; for tigecycline, susceptible (≤ 2 µg/ml) and -resistant (≥ 8 µg/ml) breakpoints are used for comparison purposes only [Jones, 1999].
b. Includes: *Acinetobacter anitratus* (eight strains), *A. baumannii* (327 strains), *A. calcoaceticus* (18 strains), *A. junii* (three strains), *A. lwoffii* (37 strains) and *Acinetobacter* spp. (91 strains).
c. Includes: *A. anitratus* (three strains), *A. baumannii* (92 strains), *A. calcoaceticus* (four strains), *A. lwoffii* (two strains) and *Acinetobacter* spp. (eight strains).

- The rank order of susceptibility among agents tested against *Klebsiella* spp. was: imipenem (99.5%) > tigecycline (97.9%) > amikacin (93.1%) > cefepime (90.4%) > levofloxacin (90.2%). Among confirmed ESBL-producing strains, only imipenem (100.0%) and tigecycline (95.6%) displayed significant activity; no other agent had ≥ 70% susceptibility.
- ESBL-confirmed *E. coli* (4.9% of tested isolates) and *Klebsiella* spp. (17.6%) displayed only 2-fold increase in their MIC_{90} values compared to the entire population of MICs for that species.
- Among imipenem-susceptible *Acinetobacter* spp. strains, tigecycline and imipenem displayed the highest and equal potencies (MIC_{90}, 2 µg/ml; 94.6% susceptible to tigecycline); only polymyxin B was more potent (MIC_{90}, ≤ 1 µg/ml; 99.0% susceptible; Table 2).
- Imipenem-non-susceptible *Acinetobacter* spp. were highly resistant (> 65%) to all agents tested with the exceptions of polymyxin B (MIC_{90}, ≤ 1 µg/ml; 99.2% susceptible) and tigecycline (MIC_{90}, 4 µg/ml; 88.1% susceptible).
- *S. maltophilia* is among the most resistant species routinely isolated by clinical laboratories; trimethoprim/sulfamethoxazole (MIC_{90}, 1 µg/ml; 97.4% susceptible) and tigecycline (MIC_{90}, 2 µg/ml; 91.5%) provided the best overall coverage for this species, with levofloxacin and polymyxin B displaying more modest susceptibility rates (86.2 and 86.1%, respectively).
- Against *B. cepacia*, those agents providing greatest activity included ceftazidime (95.2% susceptible) and trimethoprim/sulfamethoxazole (88.1%); tigecycline was only modestly active (MIC_{90}, 8 µg/ml, 69.0%).

Table 3. Activity of tigecycline tested against non-fermentative bacilli, *E. coli* and *Klebsiella* spp. (including resistant subsets).

Organism (no. tested)	$MIC_{50/90}$ (µg/ml)	Cumulative % inhibited at MIC (µg/ml) ≤0.06	0.12	0.25	0.5	1	2	4	8	16
Acinetobacter spp.										
Imipenem-susceptible (484)	0.5/2	4	19	40	56	76	95	99	100	-
Imipenem-non-susceptible (109)	1/4	0	1	2	15	50	88	99	100	-
S. maltophilia (341)	1/2	0	2	10	38	73	91	98	100	-
B. cepacia (42)	2/8	2	5	5	21	48	69	86	98	100
E. coli										
All strains (5,326)	0.12/0.25	9	53	91	>99	>99	>99	100	-	-
ESBL-confirmed strains (263)	0.25/0.5	4	33	80	99	>99	100	-	-	-
Klebsiella spp.										
All strains (2,204)	0.5/1	0	1	36	83	94	98	100	-	-
ESBL-confirmed strains (389)	0.5/2	0	1	21	63	84	96	100	-	-

CONCLUSIONS

- These findings confirm that tigecycline possesses remarkable potency against inherently resistant non-fermentative bacilli populations including *Acinetobacter* spp., *S. maltophilia* and *B. cepacia*.
- Among *E. coli* and *Klebsiella* spp., ≥ 98% of isolates were found to be susceptible at 2 µg/ml (the USA-FDA breakpoint for susceptibility) and all isolates were inhibited at 4 µg/ml. The presence of ESBL resistance mechanisms among these two species had little influence on coverage by tigecycline (no resistant strains were detected).
- Because of the inherent stability of tigecycline to the commonly occurring tetracycline resistance mechanisms, coupled with its enhanced potency and broader spectrum of activity, this novel agent represents a welcome addition to the few remaining parenteral agents active against these resistant Gram-negative pathogens.