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In Vitro Activity of Tigecycline against Multiple-Drug-Resistant, Including Pan-Resistant, Gram-Negative and Gram-Positive Clinical Isolates from Greek Hospitals

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The in vitro activities of tigecycline and selected antimicrobials were evaluated against a variety of multiple-drug-resistant clinical isolates, including extended-spectrum β-lactamase- and/or metallo-β-lactamase-producing gram-negative strains, colistin-resistant strains, vancomycin- and/or linezolid-resistant enterococci, and methicillin-resistant *Staphylococcus aureus* (MRSA). Tigecycline showed excellent activity against a collection of difficult-to-treat pathogens currently encountered in the hospital setting.

Bacterial resistance is an increasing threat to the successful treatment of both community-acquired and hospital infections (http://www.earss.rivm.nl; 9, 10, 15, 18). Thus, the development of new antimicrobial agents has been urgently needed. Tigecycline, a member of the glycylcycline class of antibiotics, provides good activity against a broad range of gram-positive and gram-negative bacteria, with the exception of *Pseudomonas aeruginosa*, *Proteus mirabilis*, and indole-positive *Proteus* spp. (1, 2). The aim of the present study was to evaluate the in vitro activities of tigecycline against a contemporary collection of multiple-drug-resistant clinical isolates, including strains that are resistant to every currently available antibiotic.

Clinical isolates collected between September 2003 and November 2005 in 17 tertiary-care hospitals in the area of Athens were analyzed. Only isolates resistant to two or more of the most commonly used antimicrobial classes for the treatment of the indicated infections were included in the study, and only one isolate per patient was accepted. Identification was performed using routine microbiologic methodologies and an automated identification system (API ID32GN and ID32E systems, bioMérieux, Marcy-l'Etoile, France). Subsequently, all strains were stored at −70°C until antimicrobial testing was performed.

MICs were determined using custom broth microdilution panels (Dade Behring, West Sacramento, CA) following the

TABLE 1. In vitro activity of tigecycline against 392 multiple-drug-resistant strains

Organism (no. of isolates)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% Susceptible[a]	No. of isolates with MIC (μg/ml) of:								
				≤0.03	0.06	0.12	0.25	0.5	1	2	4	≥8
K. pneumoniae (98)	0.5	2	96.9	0	1	4	26	37	11	16	3	0
K. pneumoniae, ESBL positive (27)	1	2	92.6	0	0	0	5	8	4	8	2	0
K. pneumoniae, MBL positive (26)	0.5	2	100	0	0	3	9	9	2	3	0	0
K. pneumoniae, ESBL and MBL positive (28)	0.5	2	100	0	0	1	7	13	2	5	0	0
K. pneumoniae, colistin resistant (15)	0.5	0.5	100	0	1	1	3	9	0	1	0	0
K. pneumoniae, minocycline resistant (28)	2	4	89.3	0	0	0	0	2	8	15	3	0
E. coli (43)	0.12	0.5	100	0	8	18	11	5	1	0	0	0
E. coli, ESBL positive (33)	0.12	0.5	100	0	8	13	8	3	1	0	0	0
E. coli, MBL positive (6)	NA[c]	NA	100	0	0	3	2	1	0	0	0	0
E. coli, minocycline resistant (10)	0.12	0.5	100	0	1	4	3	1	1	0	0	0
A. baumannii (100)	0.5	1	99	0	0	1	5	46	44	3	1	0
A. baumannii, colistin resistant (3)	NA	NA	100	0	0	0	0	2	1	0	0	0
MRSA (91)	0.25	0.25	98.9	0	0	27	58	5	0	1	0	0
E. faecium, VR[b] (60)	0.03	0.06	100	46	12	2	0	0	0	0	0	0
E. faecium, VR, linezolid resistant (5)	NA	NA	100	2	3	0	0	0	0	0	0	0

[a] Tigecycline susceptibility breakpoints used were as follows: ≤2 μg/ml for *Enterobacteriaceae* and *A. baumannii*, ≤0.5 μg/ml for *S. aureus*, and ≤0.25 μg/ml for *E. faecium*.
[b] VR, vancomycin resistant.
[c] NA, not applicable.

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TABLE 2. In vitro activities of comparators against 392 multiple-drug-resistant strains

Organism and antimicrobial agent	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)	% Susceptible[a]
K. pneumoniae (98 isolates)				
Ampicillin	>32	>32	>32–>32	0
Amoxicillin-clavulanate (2:1)	>32[b]	>32[b]	2–>32[b]	22.5
Piperacillin-tazobactam (4 μg/ml)	>128[b]	>128[b]	0.5–>128[b]	23.5
Ceftriaxone	64	>64	0.06–>64	17.3
Ceftazidime	>32	>32	8–>32	6.1
Cefepime	32	>32	0.5–>32	29.6
Imipenem	4	16	0.25–>16	59.2
Levofloxacin	8	>8	0.03–>8	19.4
Amikacin	8	32	0.5–>64	91.8
Ciprofloxacin	>32	>32	0.12–>32	11.2
Minocycline	2	8	0.5–>16	64.3
Colistin	0.5	16	0.25–128	84.7
E. coli (43 isolates)				
Ampicillin	>32	>32	>32–>32	0
Amoxicillin-clavulanate (2:1)	16[b]	>32[b]	2–>32[b]	46.5
Piperacillin-tazobactam (4 μg/ml)	1[b]	128[b]	0.5–>128[b]	72.1
Ceftriaxone	64	>64	0.06–>64	16.3
Ceftazidime	8	>32	0.25–>32	58.1
Cefepime	8	>32	0.5–>32	60.5
Imipenem	0.5	2	0.25–8	97.7
Ciprofloxacin	0.5	>32	0.03–>32	53.5
Levofloxacin	0.5	>8	0.03–>8	51.2
Amikacin	2	16	0.5–>64	90.7
Minocycline	2	8	0.5–16	76.7
Colistin	0.5	0.5	0.12–128	97.7
A. baumannii (100 isolates)				
Ampicillin-sulbactam (2:1)	16[b]	256[b]	4–>256[b]	25
Piperacillin-tazobactam (4 μg/ml)	>128[b]	>128[b]	16–>128[b]	1
Ceftriaxone	>64	>64	8–>64	3
Ceftazidime	>32	>32	8–>32	1
Cefepime	>32	>32	4–>32	1
Imipenem	16	>16	1–>16	6
Levofloxacin	8	>8	2–>8	1
Amikacin	>64	>64	8–>64	4
Minocycline	1	1	0.5–4	100
Colistin	0.5	1	0.25–>1024	97
MRSA (91 isolates)				
Levofloxacin	4	8	≤0.06–16	30.8
Vancomycin	1	1	0.5–2	100
Minocycline	4	4	≤0.25–8	96.7
Linezolid	2	2	1–4	100
E. faecium VR[c] (60 isolates)				
Penicillin	>8	>8	>8–>8	0
Ampicillin	>16	>16	>16–>16	0
Piperacillin-tazobactam (4 μg/ml)	>16[b]	>16[b]	>16–>16[b]	NA[d]
Imipenem	>16	>16	>16–>16	NA
Levofloxacin	32	>32	4–>32	0
Vancomycin	>32	>32	>32–>32	0
Minocycline	≤0.25	2	≤0.25–>8	91.7
Linezolid	2	2	1–>8	91.7
Quinupristin-dalfopristin	0.5	0.5	0.25–2	96.7

[a] Susceptibility breakpoints (μg/ml) used were as follows: penicillin, ≤8; ampicillin, ≤8; amoxicillin-clavulanate, ≤8; ampicillin-sulbactam, ≤8; piperacillin-tazobactam, ≤16; ceftriaxone, ≤8; ceftazidime, ≤8; cefepime, ≤8; imipenem, ≤4; levofloxacin, ≤1 for *Staphylococcus* spp., ≤2 for all other organisms; amikacin, ≤16; ciprofloxacin, ≤1; minocycline, ≤4; vancomycin, ≤4; colistin, ≤2; linezolid, ≤2 for *Enterococcus* spp., ≤4 for *Staphylococcus* spp.; and quinupristin-dalfopristin, ≤1. (For amoxicillin-clavulanate, ampicillin-sulbactam, and piperacillin-tazobactam, the concentration refers to the β-lactam component of the combination.)

[b] Concentration refers to the β-lactam component of the combination.

[c] VR, vancomycin resistant.

[d] NA, not available; susceptibility breakpoint for *Enterococcus* spp. in CLSI guidelines (3).

manufacturer's guidelines. Colistin, ciprofloxacin, ampicillin-sulbactam, and quinupristin-dalfopristin were not included in the microdilution panels and were tested using the Etest (AB Biodisk, Solna, Sweden). *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212 were included in all experiments for quality control. MICs falling between two values of the Etest were rounded up to the next twofold value for statistical analysis.

For *S. aureus*, methicillin resistance was determined by the cefoxitin disk test (30 μg; Bio-Rad, Marnes-La-Coquette, France), as recommended by the Clinical and Laboratory Standards Institute (CLSI) (3). The susceptibility breakpoints used were as recommended by the CLSI (3). For colistin, a breakpoint of ≤2 μg/ml was used (7), whereas for tigecycline, the breakpoints used were ≤0.5 μg/ml for *S. aureus*, ≤0.25 μg/ml for enterococci, and ≤2 μg/ml for gram-negative bacteria, as approved by the FDA (Tygacil 2005, Tigecycline package insert; Wyeth Pharmaceuticals, Philadelphia, PA).

All gram-negative isolates were screened for extended-spectrum β-lactamase (ESBL) activity using the double-disk approximation test (16). The EDTA-imipenem disk synergy test was also performed for all gram-negative isolates in order to screen them for metallo-β-lactamase (MBL) production (12). Isolates with a positive EDTA-imipenem disk synergy test were subsequently evaluated for the presence of a bla_{VIM} gene by PCR amplification, as described previously (5). PCR-restriction fragment length polymorphism analysis of the PCR amplicon with SacI restriction endonuclease was used to screen for the presence of a gene belonging to the bla_{VIM-1} cluster (6). The vancomycin resistance genotype in enterococci was identified by multiplex PCR (4). *Enterococcus faecium* ATCC 51299 (*vanB*) and a *vanA*-positive *E. faecalis* clinical strain were used as controls.

A total of 392 clinical isolates were evaluated in the present study. These included 98 strains of *Klebsiella pneumoniae* derived from bronchial secretions (30.6%), blood (23.5%), urine (18.4%), pus (15.3%), and other sources (12.2%); 100 strains of *Acinetobacter baumannii* derived from bronchial secretions (56%), pus (23%), blood (13%), and other sources (8%); 43 strains of *E. coli* derived from urine (51.1%), pus (20.9%), blood (4.7%), and other sources (23.3%); 60 strains of vancomycin-resistant *E. faecium* (VRE) derived from feces (83.3%) and other sources (16.7%); and 91 strains of methicillin-resistant *S. aureus* derived from pus (68.1%), bronchial secretions (15.4%), blood (11%), and other sources (5.5%). Among the *K. pneumoniae* isolates, 27 (27.6%) were designated ESBL producers; 26 (26.5%) showed a positive EDTA-imipenem disk synergy test, which was suggestive of MBL production; and 28 (28.6%) were designated both ESBL and MBL producers. PCR amplification for the bla_{VIM} gene was positive for all 54 *K. pneumoniae* isolates with a positive phenotypic test for MBL production. Restriction fragment length polymorphism analysis of the PCR amplicon with SacI restriction endonuclease revealed the presence of a VIM-1-like gene in all 54 of the *K. pneumoniae* isolates. Among the *E. coli* isolates, 33 (76.7%) were designated ESBL producers, whereas 6 (14%) were identified as MBL producers and were positive for a VIM-1-like gene by PCR. Molecular analysis of 60 VRE isolates identified the presence of the *vanA* gene in all isolates. The susceptibility results for all tested antimicrobials are shown in Tables 1 and 2. Tigecycline MICs against a subset of gram-negative and gram-positive isolates exhibiting specific phenotypic characteristics are listed in Table 1.

Currently, multiple-drug-resistant gram-negative bacteria remain the most problematic pathogens in Greek hospitals, especially in the intensive-care units. For *A. baumannii*, imipenem and ampicillin-sulbactam resistance rates have reached 77.5 and 58.3%, and for *K. pneumoniae*, ciprofloxacin and imipenem resistance rates are 61.1 and 29.2%, respectively. The prevalence of methicillin resistance among staphylococci is up to 68.3%, and that of vancomycin resistance among enterococci is up to 16.1% in some hospitals (http://www.mednet.gr/whonet/; last assessed February 2006). Furthermore, outbreaks of VIM-1-producing *K. pneumoniae* have been described in several hospitals (8, 11, 14). Thus, antimicrobials with activities against multiple-drug-resistant pathogens are much anticipated for everyday use in clinical practice.

Against our collection of isolates, tigecycline was very potent, inhibiting 97% of *K. pneumoniae* and 99% of *A. baumannii* isolates at a concentration of ≤2 μg/ml, as well as 99% of MRSA isolates at a concentration of ≤0.5 μg/ml. All tested *E. coli* and *E. faecium* isolates were susceptible to tigecycline. Against ESBL and/or MBL producers and against pan-resistant isolates, it was uniformly active. For these isolates, tigecycline was the only active antimicrobial currently available. It should be noted, however, that the MIC_{90} for *K. pneumoniae* isolates was higher than for the other species tested, and it was at the breakpoint of susceptibility, as approved by the FDA. Overall, tigecycline had better intrinsic activity than minocycline against gram-negative and gram-positive isolates, inhibiting almost all of the minocycline-resistant isolates, with the exception of three *K. pneumoniae* strains. Resistance to tigecycline was rare in our collection of multiple-drug-resistant isolates. Acquired resistance to this antimicrobial agent both in vitro and in vivo has been described (1, 2, 17), and it has been associated with up-regulation of chromosomally mediated efflux pumps (13).

The results of the present study suggest that tigecycline represents a significant step forward over the older semisynthetic tetracyclines, showing excellent in vitro activity against strains for which adequate therapy has been limited. It is a promising antimicrobial agent that will likely have a key role in the treatment of nosocomial infections, provided that clinical efficacy in a variety of severe infections is documented.

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