

Revised Abstract

Background: Bacterial resistance patterns vary by time and country. Surveillance studies help to identify those patterns to assist in therapeutic decisions. The Tigecycline Evaluation Surveillance Trial (TEST) is a multi-year global study that assists in the recognition of current trends in resistance on many levels. This report evaluates differences in susceptibility of bacteremia pathogens isolated in Europe from 2004 to 2008. **Methods:** 7,320 bacteremia pathogens were collected and identified from 2004-2008 at 320 sites in 24 countries in Europe. MICs for each strain were determined per EUCAST guidelines at each facility using broth microdilution. **Results:** Tigecycline MICs are recorded in the following table:

Organisms (n=7,320)	Tigecycline MICs (mg/L)		
	MIC ₅₀	MIC ₉₀	Range
<i>Acinetobacter</i> spp. (n=457)	0.25	1	≤0.008- 4
<i>Enterobacter</i> spp. (n=871)	0.5	2	0.06 - 8
<i>Enterococcus</i> spp. (n=677)	0.12	0.25	≤0.008- 0.5
<i>E. coli</i> (n=1,426)	0.12	0.25	≤0.008- 2
<i>Klebsiella</i> spp. (n=1,087)	0.5	2	0.06 - 16
<i>P. aeruginosa</i> (n=562)	8	16	0.25 - >16
<i>Serratia</i> spp. (n=295)	1	2	0.06 - 8
<i>S. agalactiae</i> (n=187)	0.03	0.12	0.015 - 0.25
<i>S. aureus</i> (n=939)	0.12	0.25	0.03 - 1
MRSA (n=230)	0.12	0.25	0.03 - 1
MSSA (n=709)	0.12	0.25	0.03 - 0.5
<i>S. pneumoniae</i> (n=474)	0.03	0.12	≤0.008 - 0.5

Conclusions: Tigecycline showed excellent *in vitro* activity against all causative bacteremic pathogens with the exception of *P. aeruginosa*. Tigecycline demonstrated MIC₉₀ values of ≤0.5mg/L against gram-positive pathogens (including resistant phenotypes) and MIC₉₀ values of ≤2mg/L against the *Enterobacteriaceae* and *Acinetobacter* spp. Tigecycline possesses potent activity against bacteremic pathogens.

Introduction

Tigecycline is a novel antimicrobial with expanded broad-spectrum activity from a new class of compounds, the glycylcyclines. Tigecycline inhibits protein synthesis by binding to the 30S ribosomal subunit. Although it is perceived to be bacteriostatic, it 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 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 active against multi-resistant *Acinetobacter* spp. Similar activity has been observed against *Enterobacteriaceae*, including extended-spectrum β-lactamase (ESBL) and *AmpC* producing strains [10]. Tigecycline has demonstrated MIC₉₀ values of ≤0.5 mg/L against methicillin-resistant *Staphylococcus aureus* (MRSA) and other gram-positive organisms [2, 4-6].

The Tigecycline Evaluation and Surveillance Trials (T.E.S.T.) program determined the *in vitro* activity of tigecycline compared to most commonly prescribed broad spectrum antibiotics against gram-positive and gram-negative species. This study was designed to evaluate the *in vitro* activity of tigecycline against bacteremic pathogens collected from European hospitals.

Materials & Methods

- Clinical isolates from hematologic sources were collected and tested between January 2004 and April 2008 from 320 sites in Europe. Isolates were identified to the species level and tested using broth microdilution at each site by the participating laboratory. All isolates were derived from blood culture specimens. Only one isolate per patient was accepted.
- Custom broth microdilution panels were supplied by MicroScan (Dade Behring Inc., Sacramento, CA, USA) and TREK (TREK Diagnostic Systems, West Sussex, England). Antimicrobial agents and concentrations tested (expressed in mg/L) were as follows: 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, MicroScan panels only); meropenem (0.12-16, MicroScan and TREK panels); linezolid (0.5-8); levofloxacin (0.06-32); minocycline (0.25-8); tigecycline (0.008-16); penicillin (0.06-8); 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, MicroScan panels only); meropenem (0.06-16, MicroScan and TREK panels); levofloxacin (0.008-8); minocycline (0.5-16); tigecycline (0.008-16) and piperacillin-tazobactam (0.06/4-128/4).
- 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; *Escherichia coli* ATCC 35218; *Haemophilus influenzae* ATCC 49247; *Haemophilus influenzae* ATCC 49766; *Staphylococcus aureus* ATCC 29213; *Streptococcus pneumoniae* ATCC 49619; *Klebsiella pneumoniae* ATCC 700603 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).

References

- Sum, P.E. and P. Petersen, *Synthesis and structure-activity relationship of novel glycylcycline derivatives leading to the discovery of GAR-936*. Biorg Med Chem Lett. 1999, 9(10): p. 1459-62.
- Abbanat, D., M. Macielag, and K. Bush, *Novel antibacterial agents for the treatment of serious gram-positive infections*. Expert Opin Investig Drugs, 2003, 12(3): p. 379-99.
- Betriu, C., et al., *In vitro activities of tigecycline (GAR-936) against recently isolated clinical bacteria in Spain*. Antimicrob Agents Chemother. 2002, 46(3): p. 892-5.
- Gates, A.C. and R.N. Jones, *Antimicrobial activity and spectrum of the new glycylcycline, GAR-936 tested against 1,203 recent clinical bacterial isolates*. Diagn Microbiol Infect Dis. 2000, 38(1): p. 19-36.
- Henwood, C.J., et al., *Antibiotic resistance among clinical isolates of Acinetobacter in the UK, and in vitro evaluation of tigecycline (GAR-936)*. J Antimicrob Chemother. 2002, 49(3): p. 479-87.
- Chopra, I., *New developments in tetracycline antibiotics: glycylcyclines and tetracycline efflux pump inhibitors*. Drug Resist Updat. 2002, 5(3-4): p. 119-25.
- Projan, S.J., *Preclinical pharmacology of GAR-936, a novel glycylcycline antibacterial agent*. Pharmacotherapy. 2000, 20(9 Pt 2): p. 219S-223S; discussion 224S-228S.
- Biedenbach, D.J., M.L. Beach, and R.N. Jones, *In vitro antimicrobial activity of GAR-936 tested against antibiotic-resistant gram-positive blood stream infection isolates and strains producing extended-spectrum β-lactamases*. Diagn Microbiol Infect Dis. 2001, 40(4): p. 173-8.
- Patel, R., et al., *In vitro activity of GAR-936 against vancomycin-resistant enterococci, methicillin-resistant Staphylococcus aureus and penicillin-resistant Streptococcus pneumoniae*. Diagn Microbiol Infect Dis. 2000, 38(3): p. 177-9.
- Petersen, P.J., et al., *In vitro and in vivo antibacterial activities of a novel glycylcycline, the 9-t-butylglycylamido derivative of minocycline (GAR-936)*. Antimicrob Agents Chemother. 1999, 43(4): p. 738-44.
- Petersen, P.J., et al., *In vitro and in vivo activities of tigecycline (GAR-936), daptomycin, and comparative antimicrobial agents against glycopeptide-intermediate Staphylococcus aureus and other resistant gram-positive pathogens*. Antimicrob Agents Chemother. 2002, 46(8): p. 2595-601.
- Clinical and Laboratory Standards Institute (CLSI). 2009. Performance Standards for Antimicrobial Susceptibility Testing: Fourteenth Information Supplement. CLSI, document M100-S18. Wayne, PA, USA.
- Tygacil®. 2005. Tigecycline FDA package insert.

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Results

Figure 1. Top ten microorganisms isolates from blood cultures in Europe (2004-2008).

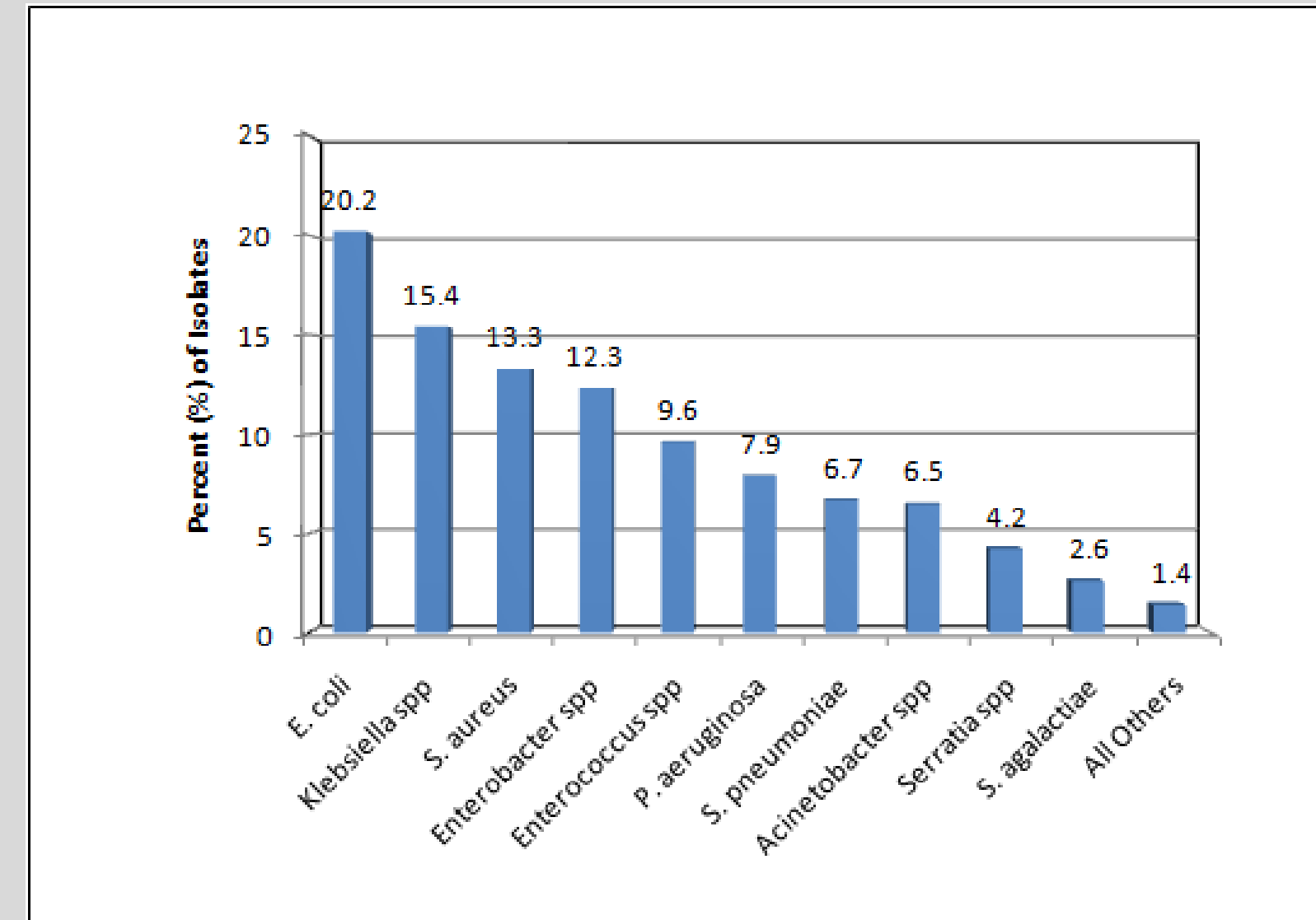


Table 1. The *in vitro* activity of tigecycline and comparative agents against *Enterobacteriaceae* isolated from blood specimens from Europe.

Organism	Drug	MIC (mg/L)		Range	%Sus ^a
		MIC ₅₀	MIC ₉₀		
<i>Enterobacter</i> spp. (n=871)	Tigecycline	0.5	2	0.06 - 8	88.5
	Amikacin	2	4	≤0.5 - >64	95.9
	AmoxClav	>32	>32	1 - >32	0.7
	Ampicillin	>32	>32	2 - >32	0.2
	Cefepime	≤0.5	8	≤0.5 - >32	73.5
	Ceftazidime	≤8	>32	≤8 - >32	0.0
	Ceftriaxone	0.5	>64	≤0.06 - >64	58.1
	Imipenem	0.5	1	≤0.06 - 8	99.1
	Levofloxacin	0.06	>8	≤0.008 - >8	82.0
	Meropenem	≤0.06	0.25	≤0.06 - 16	98.6
	Minocycline	2	8	≤0.5 - >16	80.0
PipTazo	4	128	0.25 - >128	64.6	
<i>E. coli</i> (n=1,426)	Tigecycline	0.12	0.25	≤0.008 - 2	99.8
	Amikacin	2	4	≤0.5 - >64	98.0
	AmoxClav	8	32	≤0.12 - >32	46.0
	Ampicillin	>32	>32	≤0.5 - >32	38.0
	Cefepime	≤0.5	2	≤0.5 - >32	89.5
	Ceftazidime	≤8	≤8	≤8 - >32	0.0
	Ceftriaxone	≤0.06	4	≤0.06 - >64	89.3
	Imipenem	0.25	0.5	≤0.06 - 2	100.0
	Levofloxacin	0.03	>8	≤0.008 - >8	73.5
	Meropenem	≤0.06	≤0.06	≤0.06 - 0.5	100.0
	Minocycline	1	8	≤0.5 - >16	80.6
PipTazo	1	8	0.12 - >128	91.5	
<i>Klebsiella</i> spp. (n=1,087)	Tigecycline	0.5	2	0.06 - 16	16
	Amikacin	2	8	≤0.5 - >64	>64
	AmoxClav	4	32	0.5 - >32	>32
	Ampicillin	>32	>32	4 - >32	>32
	Cefepime	≤0.5	32	≤0.5 - >32	>32
	Ceftazidime	≤8	>32	≤8 - >32	>32
	Ceftriaxone	≤0.06	>64	≤0.06 - >64	>64
	Imipenem	0.25	0.5	≤0.06 - >16	>16
	Levofloxacin	0.06	8	≤0.008 - >8	>8
	Meropenem	≤0.06	0.25	≤0.06 - >16	>16
	Minocycline	2	16	≤0.5 - >16	>16
PipTazo	2	128	≤0.06 - >128	>128	
<i>Serratia</i> spp. (n=295)	Tigecycline	1	2	0.06 - 8	87.5
	Amikacin	2	4	≤0.5 - >64	96.9
	AmoxClav	>32	>32	2 - >32	2.4
	Ampicillin	>32	>32	2 - >32	1.7
	Cefepime	≤0.5	1	≤0.5 - >32	92.2
	Ceftazidime	≤8	≤8	≤8 - >32	0.0
	Ceftriaxone	0.25	16	≤0.06 - >64	78.3
	Imipenem	0.5	1	≤0.06 - >16	97.4
	Levofloxacin	0.12	1	≤0.008 - >8	90.2
	Meropenem	≤0.06	0.2	≤0.06 - >16	97.9
	Minocycline	2	8	≤0.5 - >16	80.7
PipTazo	1	16	0.12 - >128	88.1	

^a Breakpoints as defined by EUCAST, where available, 2009; CLSI breakpoints were used where EUCAST not available. na = not available; breakpoints not defined. Tigecycline breakpoints defined by FDA (Tygacil®, 2005).

Table 2. The *in vitro* activity of tigecycline and comparative agents against *Acinetobacter* spp. and *Pseudomonas aeruginosa* isolated from blood cultures from Europe.

Organism	Drug	MIC (mg/L)		Range	%Sus
		MIC ₅₀	MIC ₉₀		
<i>Acinetobacter</i> spp. (n=457)	Tigecycline	0.25	1	≤0.008 - 4	na
	Amikacin	2	>64	≤0.5 - >64	68.7
	Cefepime	8	>32	≤0.5 - >32	61.7
	Ceftazidime	≤8	>32	≤8 - >32	52.7
	Ceftriaxone	16	>64	≤0.06 - >64	41.8
	Imipenem	0.5	>16	≤0.06 - >16	79.1
	Levofloxacin	0.25	>8	≤0.008 - >8	57.3
	Meropenem	1	>16	≤0.06 - >16	64.1
	Minocycline	≤0.5	2	≤0.5 - >16	96.7
	PipTazo	4	>128	≤0.06 - >128	59.1
	<i>P. aeruginosa</i> (n=562)	Tigecycline	8	16	0.25 - >16
Amikacin		4	16	≤0.5 - >64	87.2
Cefepime		4	32	≤0.5 - >32	76.9
Ceftazidime		≤8	32	≤8 - >32	76.7
Ceftriaxone		64	>64	1 - >64	17.1
Imipenem		1	8	0.12 - >16	82.5
Levofloxacin		0.5	>8	0.015 - >8	60.9
Meropenem		1	16	≤0.06 - >16	72.8
PipTazo		4	128	0.25 - >128	77.0

^a Breakpoints as defined by EUCAST, where available, 2009; CLSI breakpoints were used where EUCAST not available. na = not available; breakpoints not defined.

Table 3. The *in vitro* activity of tigecycline and comparative agents against gram-positive pathogens isolated from blood specimens from Europe.

Organism	Drug	MIC (mg/L)		Range	%Sus ^a
		MIC ₅₀	MIC ₉₀		
<i>S. aureus</i> , MRSA ^b (n=230)	Tigecycline	0.12	0.25	0.03 - 1	99.6
	AmoxClav	>8	>8	0.25 - >8	3.9
	Ampicillin	>16	>16	1 - >16	0.0
	Ceftriaxone	>64	>64	2 - >64	0.0
	Imipenem	4	>16	≤0.12 - >16	0.0
	Levofloxacin	8	32	0.12 - >32	7.0
	Linezolid	2	4	≤0.5 - 4	100.0
	Meropenem	8	>16	≤0.12 - >16	0.0
	Minocycline	≤0.25	0.5	≤0.25 - >8	90.4
	Penicillin	>8	>8	0.25 - >8	0.0
	PipTazo	>16	>16	0.5 - >16	8.3
Vancocycin	1	1	0.25 - 2	100.0	
<i>S. aureus</i> , MSSA ^b (n=709)	Tigecycline	0.12	0.25	0.03 - 0.5	100.0
	AmoxClav	0.5	>8	≤0.03 - 8	99.6
	Ampicillin	2	>16	≤0.06 - >16	66.8
	Ceftriaxone	2	4	0.5 - >64	100.0
	Imipenem	≤0.12	0.25	≤0.12 - 1	100.0
	Levofloxacin	0.12	0.25	≤0.06 - >32	98.0
	Linezolid	2	4	≤0.5 - 4	100.0
	Meropenem	≤0.12	0.25	≤0.12 - >16	100.0
	Minocycline	≤0.25	0.5	≤0.25 - 4	98.3
	Penicillin	4	>8	≤0.06 - >8	19.6
	PipTazo	1	1	≤0.25 - 8	100.0
Vancocycin	1	1	≤0.12 - >32	100.0	
<i>Enterococcus</i> spp. ^c (n=677)	Tigecycline	0.12	0.25	≤0.03 - 0.5	99.9
	AmoxClav	1	>8	≤0.03 - >8	69.3
	Ampicillin	1	>16	≤0.06 - >16	69.1
	Ceftriaxone	>64	>64	≤0.03 - >64	1.2
	Imipenem	4	>16	≤0.12 - >16	61.3
	Levofloxacin	4	>32	0.12 - >32	38.4
	Linezolid	2	2	≤0.5 - 4	100.0
	Minocycline	4	>8	≤0.25 - >8	54.1
	Penicillin	4	>8	≤0.06 - >8	68.8
	Vancocycin	1	2	≤0.12 - >32	93.8
	<i>S. pneumoniae</i> (n=474)	Tigecycline	0.03	0.12	≤0.008 - 0.5
AmoxClav		1	>8	≤0.03 - >8	97.9
Ampicillin		≤0.06	2	≤0.06 - 8	85.7
Azithromycin		0.12	>64	≤0.03 - >64	72.0
Ceftriaxone		≤0.03	0.5	≤0.03 - >64	90.5
Clarithromycin		0.03	>64	≤0.015 - >64	72.3
Clinfiamycin		0.06	>64	≤0.015 - >64	81.6
Erythromycin		0.12	>64	≤0.015 - >64	73.7
Imipenem		≤0.12	0.25	≤0.12 - >	