

# Antimicrobial Susceptibility of Bacteremic Pathogens in Europe: Tigecycline Evaluation Surveillance Trial (TEST) - 2004-2008

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## Revised Abstract

**Objectives:** Bacterial resistance patterns vary over both time and geography. One of the goals of surveillance studies is to identify those patterns to help guide current therapy. The Tigecycline Evaluation Surveillance Trial (TEST) is an ongoing global study that can serve to help recognize current trends in resistance on many levels. This report evaluates differences in susceptibility of bacterial pathogens isolated from the blood stream, collected in Europe from 2004 to 2008. **Methods:** 6,635 bacteremia pathogens were collected and identified from 2004-2008 at 320 hospitals 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=6,635)	Tigecycline MICs (mcg/ml)		
	MIC <sub>50</sub>	MIC <sub>90</sub>	Range
<i>Acinetobacter</i> spp (n=457)	0.25	1	≤0.008 - 4
<i>P. aeruginosa</i> (n=562)	8	16	0.25 - >16
<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
VREs (n=38)	0.06	0.12	0.015 - 0.25
<i>E. coli</i> (n=1,426)	0.12	0.25	≤0.008 - 2
<i>Klebsiella</i> spp (n=1,087)	0.5	2	0.12 - 16
ESBLs (n=250)	0.5	2	0.06 - 8
<i>Serratia</i> spp (n=295)	1	2	0.06 - 8
<i>H. influenzae</i> (n=50)	0.12	0.5	0.03 - 1
<i>S. aureus</i> (n=939)	0.12	0.25	0.03 - 0.5
MRSA (n=230)	0.12	0.25	0.03 - 0.5
<i>S. agalactiae</i> (n=187)	0.03	0.12	0.015 - 0.25
<i>S. pneumoniae</i> (n=474)	0.03	0.12	≤0.008 - 0.5

**Conclusions:** Tigecycline showed excellent inhibitory activity against all causative bacteremic pathogens with the exception of *P. aeruginosa*. Tigecycline demonstrated MIC<sub>90</sub> values of ≤0.25mcg/ml against gram-positive pathogens (including resistant phenotypes) and MIC<sub>90</sub> values of ≤2 mcg/ml against the *Enterobacteriaceae* and *Acinetobacter* spp. validate the potent inhibitory activity of TIG against these invasive 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 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 active against multi-resistant *Acinetobacter* spp., particularly *A. baumannii*, that are commonly associated with serious nosocomial infections. Similar activity has been observed against *Enterobacteriaceae*, including extended-spectrum beta-lactamase (ESBL) and AmpC producing strains [10]. Tigecycline has demonstrated MIC<sub>90</sub> values of ≤0.5 mcg/ml 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 collected from 1016 hospitals globally from 2004 to 2008. This study was designed to evaluate the in vitro activity of tigecycline against bacteremia pathogens collected from European hospitals.

## Materials & Methods

- All isolates were derived from blood culture specimens. Only one isolate per patient was accepted.
- Clinical isolates (n=6,635) were collected and tested between January 2004 and December 2008 from 320 sites in 24 European countries (Austria, Belgium, Croatia, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Norway, Poland, Portugal, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, The Netherlands, and the United Kingdom). Isolates were identified to the species level and tested using both microdilution at each site by the participating laboratory.
- Custom broth microdilution panels were supplied by MicroScan (Dade Behring Inc., Sacramento, CA, USA) with the following antimicrobial agents and concentrations (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 breakpoints established by EUCAST where applicable [15]. If no EUCAST guidelines were available for a given antibiotic, CLSI breakpoints [12] were used.
- MIC interpretive criteria for Tigecycline followed published guidelines established by the FDA where applicable [13].
- 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.
- Escherichia coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca* were screened for ESBL activity when MIC results for ceftriaxone were >1 mcg/ml using broth microdilution panels. ESBL activity was confirmed using the CLSI (2006) phenotypic confirmatory disk test (Oxoid, Ogdensburg, NY, USA) on Mueller-Hinton agar (Remel Inc., Lenexa, KS, USA) according to CLSI (2006) guidelines. ESBL presence was confirmed by testing the following antibiotic disks: cefotaxime (30-mcg), cefotaxime/clavulanic acid (30/10-mcg), ceftazidime (30-mcg), and ceftazidime/clavulanic acid (30/10-mcg). Antimicrobial disks were manufactured by Oxoid, Inc. (Ogdensburg, 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 >5 mm in the inhibition zone of the combination disk when compared to that of the cephalosporin alone.
- 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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## Results

Figure 1. The distribution of 6,635 European blood culture isolates by organism type.

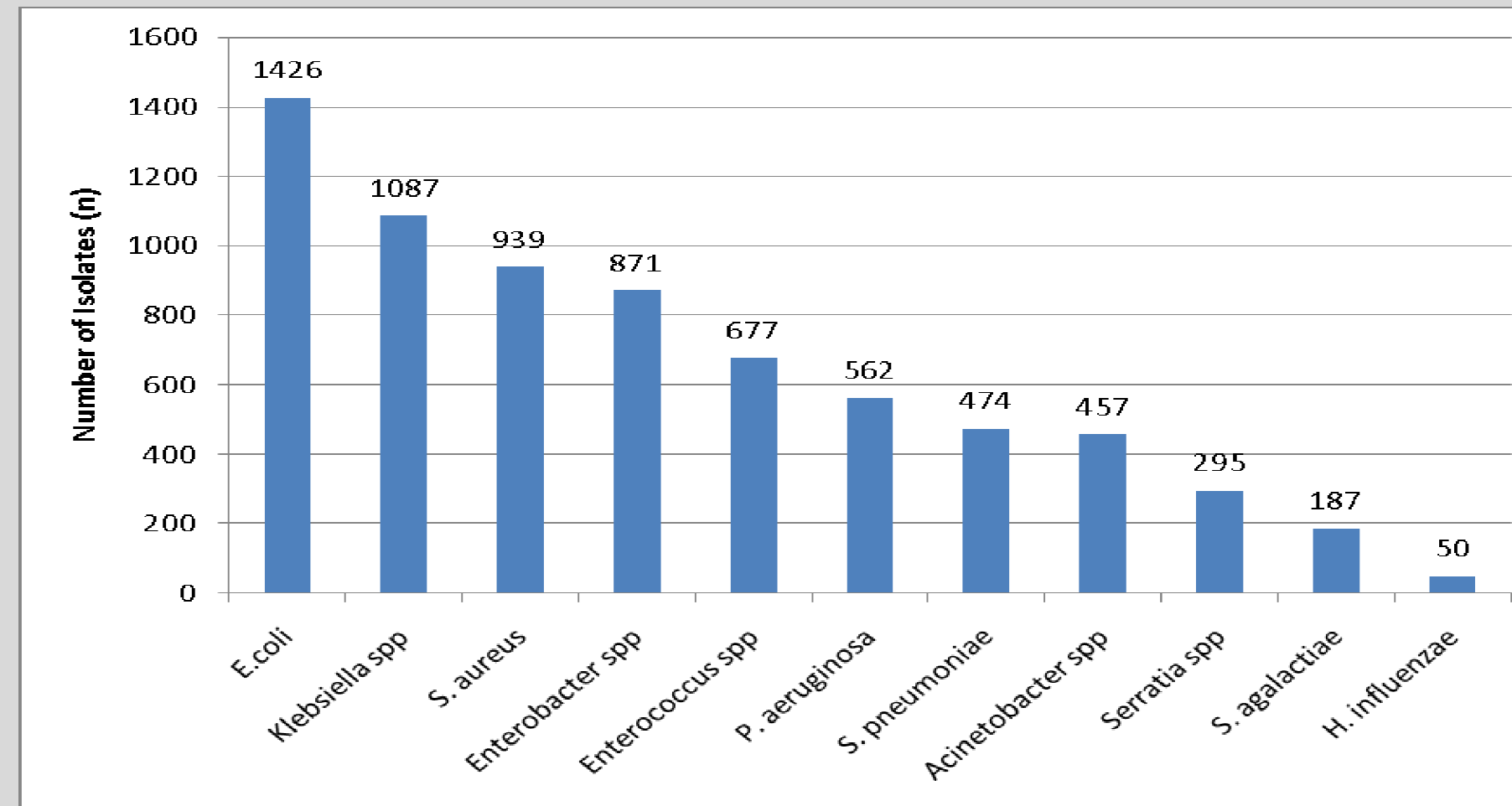


Table 1. The in vitro activity of tigecycline and comparative agents against *Enterobacteriaceae* isolated from European blood specimens.

Organism	Drug	%Sus	MIC (mcg/ml)	
			MIC <sub>50</sub>	MIC <sub>90</sub>
<i>Enterobacter</i> spp. (n=871)	Tigecycline	88.5	0.5	2
	Amikacin	95.9	2	4
	Amox/Clav	1.5	>32	>32
	Ampicillin	0.3	>32	>32
	Cefepime	73.5	≤0.5	8
	Ceftriaxone	58.1	0.5	>64
	Imipenem	99.1	0.5	1
	Levofloxacin	82	0.06	>8
	Minocycline	80	2	8
	Pip/Tazo	69.6	4	128
	<i>E. coli</i> (n=1426)	Tigecycline	99.8	0.12
Amikacin		98	2	4
Amox/Clav		71.5	8	32
Ampicillin		39.3	>32	>32
Cefepime		89.5	≤0.5	2
Ceftriaxone		89.3	≤0.06	4
Imipenem		100	0.25	0.5
Levofloxacin		73.5	0.03	>8
Minocycline		80.6	1	>8
Pip/Tazo		93.8	1	8
<i>Klebsiella</i> spp. (n=1087)		Tigecycline	88.5	0.5
	Amikacin	94.9	2	8
	Amox/Clav	72.6	4	32
	Ampicillin	0.6	>32	>32
	Cefepime	79.5	≤0.5	32
	Ceftriaxone	76	≤0.06	>64
	Imipenem	99.2	0.25	0.5
	Levofloxacin	82	0.06	8
	Minocycline	78.5	2	16
	Pip/Tazo	83	2	>128
	All ESBL producers <sup>b</sup> (n=250)	Tigecycline	87.6	0.5
Amikacin		80.4	4	16
Amox/Clav		23.6	16	32
Ampicillin		0.4	>32	>32
Cefepime		8.4	32	>32
Ceftriaxone		1.2	>64	>64
Imipenem		100	0.25	0.5
Levofloxacin		30.8	8	>8
Minocycline		58.4	4	>16
Pip/Tazo		62.8	8	>128
<i>Serratia</i> spp. (n=295)		Tigecycline	87.5	1
	Amikacin	96.9	2	4
	Amox/Clav	3.7	>32	>32
	Ampicillin	3.1	>32	>32
	Cefepime	92.2	≤0.5	1
	Ceftriaxone	78.3	0.25	16
	Imipenem	97.4	0.5	1
	Levofloxacin	90.2	0.12	1
	Minocycline	80.7	2	8
	Pip/Tazo	90.2	1	16

<sup>a</sup> Interpretive criteria as defined by EUCAST, where available and CLSI breakpoints, where available. If no EUCAST breakpoints exist, na = not available.

<sup>b</sup> ESBL-Extended Spectrum Beta-lactamase producing strain; includes *E. coli*, *K. pneumoniae* and *K. oxytoca*.

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

Organism	Drug	%Sus	MIC (mcg/ml)		
			MIC <sub>50</sub>	MIC <sub>90</sub>	
<i>Acinetobacter</i> spp. (n=457)	Tigecycline	na	0.25	1	
	Amikacin	68.7	2	>64	
	Cefepime	61.7	8	>32	
	Ceftriaxone	41.8	16	>64	
	Imipenem	79.1	0.5	>16	
	Levofloxacin	57.3	0.25	>8	
	Minocycline	96.7	≤0.5	2	
	Pip/Tazo	59.1	4	>128	
	<i>P. aeruginosa</i> (n=562)	Tigecycline	na	8	16
		Amikacin	87.2	4	16
		Cefepime	76.9	4	32
Ceftriaxone		17.1	64	>64	
Imipenem		82.5	1	8	
Levofloxacin		60.9	0.5	8	
Minocycline		na	16	>16	
Pip/Tazo		88.4	4	128	

<sup>a</sup> Interpretive criteria as defined by EUCAST, where available and CLSI breakpoints, where available. If no EUCAST breakpoints exist, na = not available.

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

Organism	Drug	%Sus	MIC (mcg/ml)		
			MIC <sub>50</sub>	MIC <sub>90</sub>	
<i>S. aureus</i> , MRSA <sup>a</sup> (n=230)	Tigecycline	100	0.12	0.25	
	Amox-Clav	3.9	>8	>8	
	Ampicillin	0	>16	>16	
	Ceftriaxone	0	>64	>64	
	Imipenem	0	4	>16	
	Levofloxacin	7	8	32	
	Linezolid	100	2	4	
	Minocycline	90.4	≤0.25	0.5	
	Penicillin	0	>8	>8	
	Pip-Tazo	8.3	>16	>16	
	Vancomycin	100	1	1	
<i>S. aureus</i> , MSSA (n=709)	Tigecycline	100	0.12	0.25	
	Amox-Clav	90.6	0.5	1	
	Ampicillin	66.6	2	>16	
	Ceftriaxone	100	2	4	
	Imipenem	100	≤0.12	0.25	
	Levofloxacin	98	0.12	0.25	
	Linezolid	100	2	4	
	Minocycline	98.3	≤0.25	≤0.25	
	Penicillin	64.6	4	>8	
	Pip-Tazo	100	1	1	
	Vancomycin	100	1	1	
<i>Enterococcus</i> spp. <sup>c</sup> (n=677)	Tigecycline	99.9	0.12	0.25	
	Ampicillin	69.6	1	>16	
	Levofloxacin	38.4	4	>32	
	Linezolid	100	2	2	
	Minocycline	54.1	4	>8	
	Penicillin	68.8	4	>8	
	Vancomycin	93.8	1	2	
	<i>S. agalactiae</i> (n=187)	Tigecycline	100	0.03	0.12
		Ampicillin	100	0.12	0.12
		Levofloxacin	98.4	0.5	1
		Linezolid	100	1	1
Penicillin		100	≤0.06	0.12	
Vancomycin		100	0.5	0.5	

<sup>a</sup> Interpretive criteria as defined by EUCAST, where available and CLSI breakpoints, where available. If no EUCAST breakpoints exist, na = not available.

<sup>b</sup> Methicillin phenotype based upon ceftioxin 30 mcg disk results; β-lactam susceptibilities based on methicillin phenotype.

<sup>c</sup> Tigecycline FDA breakpoints for enterococci are approved for vancomycin-susceptible *E. faecalis*; only, susceptibilities for all other enterococci are entered for comparison purposes only [14].

Table 4. The in vitro activity of tigecycline and comparative agents against fastidious gram-positive pathogens isolated from European blood specimens.

Organism	Drug	%Sus	MIC (mcg/ml)	
			MIC <sub>50</sub>	MIC <sub>90</sub>
<i>S. pneumoniae</i> (n=474)	Tigecycline	na	0.03	0.12
	Ceftriaxone	90.5	≤0.03	0.5
	Imipenem	99.5	≤0.12	0.25
	Levofloxacin	99.8	0.5	1
	Linezolid	100	≤0.5	1
	Penicillin	76.6	≤0.06	1
	Vancomycin	100	0.25	0.5
	<i>Haemophilus influenzae</i> (n=50)	Tigecycline	na	0.25
Amox/Clav		100	0.25	0.5
Ampicillin		86	≤0.5	32
Ceftriaxone		96	≤0.06	≤0.06
Imipenem		100	0.5	1
Levofloxacin		100	0.015	0.03
Minocycline		92	≤0.5	1
Pip-Tazo		100	≤0.06	≤0.06

<sup>a</sup> Interpretive criteria as defined by EUCAST, where available and CLSI breakpoints, where available. If no EUCAST breakpoints exist, na = not available.

## Conclusions

- Tigecycline's MIC<sub>50</sub>/MIC<sub>90</sub> values against *Acinetobacter* spp of 0.25 and 1 mcg/ml, respectively, were the lowest of all comparator agents in this study.
- Against gram-positive organisms, including resistant phenotypes, tigecycline showed excellent in vitro activity. All strains were inhibited at ≤0.25 mcg/ml and 100% of all *S. aureus* and *Enterococcus* spp. were susceptible to tigecycline.
- Although % susceptible figures for some organism groups (*Enterobacter* spp., and ESBL-producing *E. coli* and *Klebsiella* spp.) appear somewhat lower in this analysis than in previous studies, this is more due to the one-dilution lower susceptibility breakpoint selected by EUCAST than to actual quantitative differences in MICs.