

Tigecycline Evaluation Surveillance Trial (T.E.S.T.) Program - European In Vitro Antibacterial Activity Against Selected Species of *Enterobacteriaceae*

#P 816

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REVISED ABSTRACT

Background: Rapid increasing resistance in nosocomial pathogens has always been a challenge for clinicians and hospital infection control. Tigecycline, a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent expanded broad spectrum activity against most species of *Enterobacteriaceae* as well as gram-positive, atypicals and anaerobes. The T.E.S.T. program determined the in vitro activity of tigecycline compared to amikacin, ampicillin, amoxicillin/clavulanic acid, imipenem, cefepime, ceftazidime, ceftriaxone, levofloxacin, minocycline and piperacillin/tazobactam against members of *Enterobacteriaceae* (mainly *E. coli*, *Klebsiella* spp., *Enterobacter* spp. and *Serratia* spp.) collected from 15 hospitals in Germany, France, Italy, Spain, Hungary, Latvia, Poland, Switzerland, Netherlands and United Kingdom. **Methods:** A total of 2,015 clinical isolates were identified to the species level at each participating site and confirmed by the central laboratory. Isolates were collected throughout 2004. Minimum Inhibitory Concentration (MICs) were determined by the local laboratory using broth microdilution panels from Dade Behring MicroScan and interpreted according to CLSI guidelines. **Results:** The in vitro activity of all the broad spectrum antimicrobial agents remain highly active against *Enterobacteriaceae* in Europe. The susceptibility rates for amikacin, cefepime, ceftazidime, ceftriaxone, imipenem, levofloxacin, minocycline and piperacillin/tazobactam are 98.1%, 92.3%, 86.7%, 86.2%, 99.4%, 89.3%, 83% and 90.2%, respectively. Tigecycline's activity was similar to the most effective antimicrobial agent, imipenem (99.4% susceptibility) with a MIC₅₀/MIC₉₀ of 0.25/1 mcg/ml against all strains of *Enterobacteriaceae*. The frequency of ESBL production among *K. pneumonia*, *K. oxytoca*, and *E. coli* was found to be 15.3%, 8.6% and 3.5%, respectively. Tigecycline inhibited 100% of all ESBL producing *E. coli*, *K. oxytoca* and *K. pneumoniae* at MICs of 0.5 mcg/ml, 4 mcg/ml and 8 mcg/ml, respectively. Approximately 30% of *Enterobacter* spp and 9% of *Serratia marcescens* presented resistance to third generation cephalosporins (ceftazidime and ceftriaxone) suggestive of AmpC-type resistance. **Conclusion:** Most of the broad spectrum antimicrobial agents still remain active against European representatives of *Enterobacteriaceae*. Tigecycline's in vitro activity was comparable to the activities of all broad spectrum antimicrobials with greater activity against ESBL and AmpC producing strains. The presented data suggest that tigecycline may be an effective and reliable therapeutic option against both susceptible strains of *Enterobacteriaceae* and drug resistant strains.

INTRODUCTION

Tigecycline is a novel antimicrobial with an expanded broad-spectrum of activity from a new class of compounds, glycylcyclines. Tigecycline inhibits protein synthesis by binding to the 30S ribosomal subunit. Although it is perceived to be bacteriostatic, its anti-bacterial activity is significant and 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 10⁻⁹ [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]. This broad spectrum activity has been demonstrated against gram-negative pathogens, even extended-spectrum β-lactamase producing *E. coli* and *Klebsiella pneumoniae* [10, 12]. This study was designed to better define tigecycline activity in a large diverse population of clinical isolates.

This study compared the activity of tigecycline with other agents against *Enterobacteriaceae* including *Escherichia coli*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Klebsiella pneumoniae* and *Serratia marcescens* from hospitals across Europe.

MATERIALS & METHODS

- All isolates were derived from blood, respiratory tract, urine (no more than 25% of all isolates), skin, wound, fluids and few other defined sources. Only one isolate per patient was accepted.
- Clinical isolates were collected tested between January 2004 - December 2004 from 15 study centers across 10 countries in Europe.
- Escherichia coli* and *Klebsiella pneumoniae* were screened and confirmed for ESBL activity according to CLSI guidelines (Table 2A, M100-S14) [13].
- ESBL activity was confirmed by testing the following antibiotic disks: cefotaxime (30 mcg), cefotaxime/clavulanic acid (30/10 mcg) and ceftazidime (30 mcg), ceftazidime/clavulanic acid (30/10 mcg). Antibiotic disks were manufactured by Oxoid Inc. Ogdensburg, New York. Mueller-Hinton agar used in testing was manufactured by Remel Inc. Lenexa, Kansas.
- An organism is interpreted as producing an ESBL if there is an increase of ≥ 5mm in the inhibition zone of the combination disc when compared to that of the cephalosporin alone: cefotaxime/clavulanic acid - cefotaxime ≥ 5 mm or ceftazidime/clavulanic acid - ceftazidime ≥ 5 mm.
- Antimicrobial agents tested with concentrations (expressed in mcg/ml) were: amoxicillin/clavulanic acid (0.12-32); piperacillin/tazobactam (0.06-128); levofloxacin (0.008-8); ceftriaxone (0.06-64); cefepime (0.5-32); amikacin (0.5-64); minocycline (0.5-16); ceftazidime (8-32); tigecycline (0.008-16); imipenem (0.06-16). MIC interpretive criteria followed published guidelines established by the CLSI where applicable [15]. Tigecycline tentative breakpoints (in units of mcg/mL) are defined as susceptible ≤ 2; intermediate = 4; and resistant ≥ 8.
- Isolates were identified to genus and species by the local laboratory. Each site tested the isolates using broth microdilution.
- Quality control of antibiotic disks followed manufactures guidelines (Oxoid) using the following ATCC strains: *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922.
- 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).

ACKNOWLEDGEMENTS

This study was supported by a grant from Wyeth Pharmaceuticals. We gratefully acknowledge contributions to the T.E.S.T. study from the following participating institutions: C.H.U. Cote de Nacre, France; Hopital Cardiologique, France; Institute of Micro Univ. of Ancona Med. School, Italy; University of Catania, Italy; University Hospital Geneva, Switzerland; Hospital General Universitario Gregorio Marañon, Spain; Kings College, UK; Unit of Hygiene - University of Heidelberg, Germany; P. Stradina Clinical University Hospital, Latvia; Univ. Hospital of Freiburg, Germany; Fejer Megyei ANTSZ, Hungary; Isala Kliniek - LMM, The Netherlands; National Institute of Public Health, Poland; Institute for Med Microbiology, Germany and Universitätsklinikum Aachen, Germany

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RESULTS

Table 1. List of countries and number of investigative sites that contributed to T.E.S.T. program.

Country	Investigative Sites
France	2
Germany	4
Hungary	1
Italy	2
Latvia	1
Poland	1
Spain	1
Switzerland	1
The Netherlands	1
United Kingdom	1
Total	15

Table 2. In vitro activity of tigecycline and comparative agents against 2,015 strains of *Enterobacteriaceae*.

Organism Name	Drug	MICs (mcg/mL)	
		MIC ₅₀	MIC ₉₀
<i>Enterobacteriaceae</i> (n=2,015)	Tigecycline	0.25	1
	Amikacin	2	<0.5->64
	Amox-Clav	8	>32
	Cefepime	<0.5	4
	Ceftazidime	<8	32
	Ceftriaxone	<0.06	32
	Imipenem	0.5	1
	Levofloxacin	0.06	4
	Minocycline	2	8
	Pip-Tazo	1	16

Table 3. In vitro activity of tigecycline and comparators against 2,015 strains of *Enterobacteriaceae* showing frequency distribution and cumulative percent inhibited (%) at each MIC (mcg/ml).

MIC	<0.008	0.015	0.03	<0.06	0.12	0.25	<0.5	0.5	1	2	4	<8	8	>8	>16	>32	>64	>128
Tigecycline	4	91	473	544	597	164	70	67	5									
Amikacin	0.2	4.7	28.2	55.2	84.8	93	96.4	99.8	100									
Amox-Clav			0.4	8	26.9	75.7	90.8	95.7	98.1	98.5	98.8	100						
Cefepime			0.1	0.5	3.3	31.5	46.7	59.6	68.5	75.2	100							
Ceftazidime			1647	81	56	57		38	35	24	97							
Ceftriaxone			81.7	84.8	87.5	90.4		92.3	94	95.2	100							
Ceftriaxone	1154	254	127	74	18	34	25	51	44	36	50	148						
Imipenem	57.3	69.9	76.2	79.9	80.7	82.4	83.7	86.2	88.4	90.2	92.7	100						
Levofloxacin	6	242	651	488	118	105		86	82	44	43	58	116					
Minocycline	0.3	12.3	44.6	68.7	74.6	79.8		84.1	87.1	89.3	91.5	94.2	100					
Pip-Tazo			1	2	69	318	715	434	120	96	63	41	30	52	74			

Table 4. In vitro activity of tigecycline and comparative agents against selected representatives of *Enterobacteriaceae*.

Organism Name	Drug ^a	MICs (mcg/mL)	
		%SUS	%INT
<i>Enterobacter aerogenes</i> (n=80)	Tigecycline	97.5	2.5
	Amikacin	91.3	6.2
	Amox-Clav	10	3.7
	Cefepime	86.3	1.2
	Ceftazidime	61.3	6.2
	Ceftriaxone	70	11.2
	Imipenem	100	0
	Levofloxacin	83.8	1.2
	Minocycline	83.8	9.9
	Pip-Tazo	82.5	11.2

Organism Name	Drug ^a	MICs (mcg/mL)	
		%SUS	%INT
<i>Enterobacter cloacae</i> (n=292)	Tigecycline	95.9	4.1
	Amikacin	98.3	0
	Amox-Clav	5.1	0.3
	Cefepime	93.5	1.4
	Ceftazidime	67.7	8.9
	Ceftriaxone	73.3	8.9
	Imipenem	99.3	0
	Levofloxacin	94.2	0.7
	Minocycline	87.3	8.9
	Pip-Tazo	76.4	11.3
<i>Escherichia coli</i> (n=371)	Tigecycline	99.7	0.3
	Amikacin	99.5	0
	Amox-Clav	80.9	12.1
	Cefepime	96.2	0.5
	Ceftazidime	96.2	0.8
	Ceftriaxone	95.4	0.3
	Imipenem	99.5	0
	Levofloxacin	85.2	2.7
	Minocycline	82.5	11.3
	Pip-Tazo	96.8	0.5
<i>Klebsiella pneumoniae</i> (n=254)	Tigecycline	90.6	8.6
	Amikacin	97.6	0
	Amox-Clav	73.2	12.6
	Cefepime	85.8	3.9
	Ceftazidime	84.3	1.1
	Ceftriaxone	81.1	4.3
	Imipenem	99.6	0
	Levofloxacin	91.3	2
	Minocycline	76.4	7.9
	Pip-Tazo	88.6	3.5
<i>Klebsiella oxytoca</i> (n=116)	Tigecycline	97.4	2.6
	Amikacin	97.4	0.9
	Amox-Clav	85.3	6.9
	Cefepime	91.4	2.6
	Ceftazidime	92.2	1.7
	Ceftriaxone	87.9	6
	Imipenem	99.1	0
	Levofloxacin	92.2	2.6
	Minocycline	89.7	6
	Pip-Tazo	90.5	0.9
<i>S. marcescens</i> (n=148)	Tigecycline	98.6	0.7
	Amikacin	97.3	0.7
	Amox-Clav	2.7	2.7
	Cefepime	95.9	0
	Ceftazidime	89.8	1.4
	Ceftriaxone	89.2	4
	Imipenem	98.6	0
	Levofloxacin	92.6	2
	Minocycline	90.5	8.8
	Pip-Tazo	93.2	3.4

^aBreakpoints as defined by NCCLS where applicable (M100-S14), 2004. na = not applicable. Tigecycline breakpoints defined as: susceptible ≤ 2; intermediate = 4; and resistant ≥ 8.

Table 5. In vitro activity of tigecycline and comparative agents against extended-spectrum beta-lactamase producing *Enterobacteriaceae*.

Organism Name	Drug ^a	MICs (mcg/mL)	
		%SUS	%INT
<i>Escherichia coli</i> , ESBL (n=13)	Tigecycline	100	0
	Amikacin	82.3	0
	Amox-Clav	30.8	30.7
	Cefepime	30.8	7.7
	Ceftazidime	30.8	15.4
	Ceftriaxone	15.4	7.7
	Imipenem	100	0
	Levofloxacin	53.8	0
	Minocycline	61.5	23.1
	Pip-Tazo	100	0

Organism Name	Drug ^a	MICs (mcg/mL)	
		%SUS	%INT
<i>Klebsiella pneumoniae</i> , ESBL (n=39)	Tigecycline	82.1	15.3
	Amikacin	89.7	0
	Amox-Clav	25.6	43.6
	Cefepime	41	12.8
	Ceftazidime	20.5	2.6
	Ceftriaxone	23.1	20.5
	Imipenem	97.4	0
	Levofloxacin	66.7	2.5
	Minocycline	46.2	12.8
	Pip-Tazo	59	12.8
<i>Klebsiella oxytoca</i> , ESBL (n=10)	Tigecycline	80	20
	Amikacin	70	10
	Amox-Clav	20	40
	Cefepime	30	10
	Ceftazidime	30	10
	Ceftriaxone	0	60
	Imipenem	100	0
	Levofloxacin	70	10
	Minocycline	60	40
	Pip-Tazo	60	40
All ESBL producers <i>E. coli</i> , <i>K. pneumoniae</i> , and <i>K. oxytoca</i> (n=62)	Tigecycline	85.5	12.9
	Amikacin	87.1	1.6
	Amox-Clav	25.8	40.3
	Cefepime	37.1	11.3
	Ceftazidime	24.2	6.4
	Ceftriaxone	17.7	24.2
	Imipenem	98.4	0
	Levofloxacin	64.5	3.2
	Minocycline	51.6	12.9
	Pip-Tazo	67.7	8.1

^aBreakpoints as defined by NCCLS where applicable (M100-S14), 2004. na = not applicable. Tigecycline breakpoints defined as: susceptible ≤ 2; intermediate = 4; and resistant ≥ 8.

Table 6. In vitro activity of tigecycline and comparators against 62 strains of ESBL producing *E. coli*, *K. pneumoniae* and *K. oxytoca* showing frequency distribution and cumulative percent inhibited (%) at each MIC (mcg/ml).

MIC	0.015	0.03	0.06	0.12	0.25	<0.5	0.5	1	2	4	<8	8	>8	>16	>32	>64	>128
Tigecycline			5	16	20	8	4	8	1								
Amikacin			8.1	33.9	66.1	79	85.5	96.4	100								
Amox-Clav					9.7	30.6	45.2	69.4	87.1	88.7	90.3	100					
Cefepime						3	13	25	14	7							