P0323

In vitro activity of tigecycline against β-lactam-resistant *Enterobacteriaceae* isolates collected in European countries as part of the Tigecycline European Surveillance Trial (TEST) in 2014

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Revised Abstract

Background: TEST monitors the *in vitro* activity of the glycylcycline tigecycline and comparator antimicrobial agents against clinical isolates collected in Europe. This study reports the *in vitro* activity of tigecycline against a subset of ESBL-producing and carbapenem non-susceptible *Enterobacteriaceae* isolates collected in 2014. Methods: Non-duplicate clinical isolates were collected from defined infection sites and identified to the species level. Antibiotic susceptibility testing was performed by CLSI broth microdilution by the local laboratory using supplied panels and interpreted using EUCAST breakpoints. Confirmation of meropenem MIC values and extended-spectrum-β-lactamase (ESBL) activity was performed at a central laboratory. A subset of *Enterobacteriaceae* isolates that tested with meropenem MIC values ≥ 2 mg/L and/ or were confirmed as ESBL producers were screened for the presence of genes encoding ESBLs, AmpC β-lactamases, serine carbapenemases (KPC, OXA-48, GES), and metallo-β-lactamases (MBL). Results: 8,190 *Enterobacteriaceae* were collected from sites in 19 European countries. The overall collection was 79.4% susceptible to cefepime, 98.1% susceptible to meropenem, and 92.3% susceptible to tigecycline. A subset of 1,154 isolates (634 *Klebsiella pneumoniae*, 455 *Escherichia coli*, 46 *Enterobacter* spp. and 19 other *Enterobacteriaceae*) were molecularly characterized for β-lactamase genes. 94.7% of characterized isolates, including those that produced MBLs and additional β-lactamases, were inhibited by ≤ 2 mg/L tigecycline. The tigecycline MIC distribution against characterized isolates and cumulative percent inhibited at each MIC value are shown below.

	Tigecycline MIC (mg/L)								
Enzyme content[a]	**≤0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**≥8**	**N**
ESBL	40	193	184	192	106	59	30	4	803
ESBL + AmpC		1	1	2			3		7
AmpC	1	1	1	5	4		2		14
KPC +/- ESBL +/- AmpC		3	14	43	86	40	10	4	200
OXA-48 +/- ESBL +/- AmpC	1		15	17	11	9	6		59
GES Cpase			2						2
MBL +/- ESBL +/- AmpC[b]		3	12	13	11	4	1		44
No ESBL, AmpC or Cpase identified	3	2	6	3	3	2	1		20
Total (N)	45	203	235	275	221	114	53	8	1154
% Cumulative Inhibited	3.9	21.5	41.9	65.7	84.8	94.7	99.3	100	

[a]Includes isolates that also carry original spectrum β-lactamases. Cpase, carbapenemase
[b]Includes three isolates carrying MBLs and KPC and one isolate carrying an MBL and OXA-48.

Conclusions: Tigecycline had good *in vitro* activity against *Enterobacteriaceae* that carried one or more β-lactamases, including combinations of ESBLs and carbapenemases. Tigecycline is one of the few currently available antimicrobial agents with significant activity against difficult-to-treat *Enterobacteriaceae* such as those producing MBLs.

Introduction

TEST monitors the *in vitro* activity of the glycylcycline tigecycline and comparator antimicrobial agents against clinical isolates collected in Europe. This study reports the *in vitro* activity of tigecycline against subsets of β-lactam-non-susceptible, ESBL- and carbapenemase-producing *Enterobacteriaceae* isolates collected in 19 European countries in 2014.

Materials & Methods

- Non-duplicate clinical isolates were collected from defined infection sites and identified to the species level. Antibiotic susceptibility testing was performed by CLSI broth microdilution [1] by the local laboratory using supplied panels and interpreted using EUCAST breakpoints [2].
- Confirmation of meropenem MIC values and extended-spectrum-β-lactamase (ESBL) activity was performed at a central laboratory.
- A subset of *Enterobacteriaceae* isolates that tested with meropenem MIC values ≥ 2 mg/L and/ or were confirmed as ESBL producers by Kirby Bauer combination clavulanic acid disk testing [3] were screened for the presence of genes encoding ESBLs (SHV, TEM, CTX-M, VEB, PER, GES), AmpC β-lactamases (ACC, ACT, CMY, DHA, FOX, MIR, MOX), serine carbapenemases (KPC, OXA-48, GES), and metallo-β-lactamases (NDM, VIM, IMP, SPM) by multiplex PCR assays and sequencing [4].

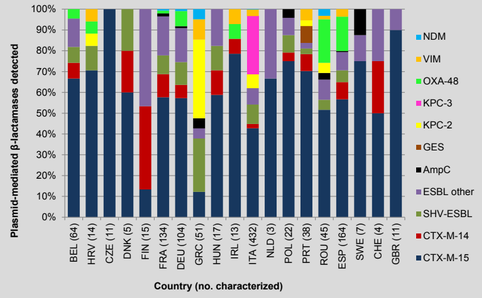
Results

Table 1. *In vitro* activity of tigecycline and comparator agents against *Enterobacteriaceae* isolates collected in European countries in 2014.

Region/ country (no. of isolates tested)	Antimicrobial agent	MIC (mg/L) MIC_{50}	MIC_{90}	Range	MIC Interpretation (%) Susceptible	Intermediate	Resistant
Europe (8,190)	TGC	0.25	1	≤0.008 - >8	92.3	5.4	2.3
	CAZ	≤1	>16	≤1 - >16	71.9	7.8	20.3
	TZP	2	64	≤0.06 - >128	81.7	4.0	14.3
	MEM	≤0.06	0.12	≤0.06 - >16	98.1	0.5	1.4
	LVX	0.06	8	≤0.008 - >8	79.4	1.8	18.8
	AMK	2	4	≤0.5 - >64	97.3	1.7	1.0
Belgium (653)	TGC	0.5	1	0.03 - 4	92.3	5.5	2.1
	CAZ	≤1	>16	≤1 - >16	74.2	5.5	20.3
	TZP	2	64	≤0.06 - >128	79.5	4.0	16.5
	MEM	≤0.06	0.12	≤0.06 - 2	100	0.0	0.0
	LVX	0.06	8	≤0.008 - >8	81.0	1.5	17.5
	AMK	2	4	≤0.5 - >64	98.5	0.9	0.6
Croatia (83)	TGC	0.5	2	0.06 - 4	86.8	10.8	2.4
	CAZ	≤1	>16	≤1 - >16	60.2	4.8	34.9
	TZP	2	128	0.5 - >128	72.3	3.6	24.1
	MEM	≤0.06	0.12	≤0.06 - >16	95.2	3.6	1.2
	LVX	0.06	>8	0.015 - >8	78.3	2.4	19.3
	AMK	4	8	1 - >64	95.2	3.6	1.2
Czech Republic (85)	TGC	0.5	1	0.06 - 4	90.6	4.7	4.7
	CAZ	≤1	>16	≤1 - >16	67.1	10.6	22.4
	TZP	2	32	0.5 - 128	81.2	4.7	14.1
	MEM	≤0.06	0.12	≤0.06 - 1	100	0.0	0.0
	LVX	0.06	4	0.015 - >8	84.7	2.4	12.9
	AMK	2	8	≤0.5 - 16	96.5	3.5	0.0
Denmark (169)	TGC	0.25	1	0.03 - >8	91.7	6.5	1.8
	CAZ	≤1	4	≤1 - >16	84.0	6.5	9.5
	TZP	1	16	0.25 - >128	88.8	1.8	9.5
	MEM	≤0.06	0.12	≤0.06 - 1	100	0.0	0.0
	LVX	0.03	0.5	≤0.008 - >8	94.1	1.2	4.7
	AMK	2	4	≤0.5 - 4	100	0.0	0.0
Finland (167)	TGC	0.25	1	0.06 - 4	91.6	6.0	2.4
	CAZ	≤1	2	≤1 - >16	88.0	7.2	4.8
	TZP	1	8	0.12 - >128	92.2	1.8	6.0
	MEM	≤0.06	0.12	≤0.06 - 1	100	0.0	0.0
	LVX	0.06	4	≤0.008 - >8	85.6	2.4	12.0
	AMK	2	4	≤0.5 - 8	100	0.0	0.0
France (1,227)	TGC	0.25	1	0.03 - 8	93.3	4.2	2.5
	CAZ	≤1	>16	≤1 - >16	70.4	6.4	23.2
	TZP	2	32	≤0.06 - >128	80.4	5.1	14.5
	MEM	≤0.06	0.12	≤0.06 - 16	99.7	0.2	0.1
	LVX	0.06	8	≤0.008 - >8	84.1	2.2	13.7
	AMK	2	4	≤0.5 - >64	98.8	0.8	0.4
Germany (1,423)	TGC	0.25	1	0.03 - 4	92.5	5.2	2.3
	CAZ	≤1	16	≤1 - >16	79.0	6.0	15.0
	TZP	2	32	≤0.06 - >128	86.4	3.1	10.5
	MEM	≤0.06	0.12	≤0.06 - >16	99.7	0.1	0.2
	LVX	0.06	4	≤0.008 - >8	86.2	1.8	12.0
	AMK	2	4	≤0.5 - >64	99.0	0.5	0.5
Greece (80)	TGC	0.5	1	0.06 - 8	91.3	6.3	2.5
	CAZ	≤1	>16	≤1 - >16	66.3	1.3	32.5
	TZP	2	>128	0.5 - >128	67.5	2.5	30.0
	MEM	≤0.06	1	≤0.06 - >16	90.0	1.3	8.8
	LVX	0.06	>8	0.015 - >8	78.8	1.3	20.0
	AMK	2	8	≤0.5 - >64	91.3	5.0	3.7
Hungary (84)	TGC	0.25	1	0.12 - 2	92.9	7.1	0.0
	CAZ	≤1	>16	≤1 - >16	60.7	8.3	31.0
	TZP	2	64	0.5 - >128	79.8	3.6	16.7
	MEM	≤0.06	0.12	≤0.06 - 0.25	100	0.0	0.0
	LVX	0.06	8	0.015 - >8	75.0	2.4	22.6
	AMK	2	8	1 - >64	96.4	2.4	1.2
Ireland (241)	TGC	0.25	1	0.03 - 4	94.2	3.7	2.1
	CAZ	≤1	16	≤1 - >16	78.6	6.8	14.5
	TZP	2	64	≤0.06 - >128	81.7	5.0	13.3
	MEM	≤0.06	0.12	≤0.06 - 1	100	0.0	0.0
	LVX	0.06	2	0.015 - >8	88.4	2.5	9.1
	AMK	2	4	1 - 16	99.6	0.4	0.0
Italy (1,325)	TGC	0.5	2	≤0.008 - 8	89.9	7.6	2.5
	CAZ	≤1	>16	≤1 - >16	63.5	7.0	29.5
	TZP	2	128	≤0.06 - >128	76.9	4.8	18.3
	MEM	≤0.06	0.5	≤0.06 - >16	92.5	1.1	6.3
	LVX	0.12	>8	≤0.008 - >8	65.4	1.1	33.5
	AMK	2	8	≤0.5 - >64	91.8	5.5	2.7
Netherlands (182)	TGC	0.25	1	0.12 - 4	97.3	2.2	0.6
	CAZ	≤1	4	≤1 - >16	87.9	2.8	9.3
	TZP	1	4	0.12 - >128	93.4	1.1	5.5
	MEM	≤0.06	0.12	≤0.06 - 1	100	0.0	0.0
	LVX	0.03	0.5	0.015 - >8	96.7	0.6	2.8
	AMK	2	4	≤0.5 - 16	99.4	0.6	0.0
Poland (159)	TGC	0.5	2	0.06 - 4	89.3	7.6	3.1
	CAZ	≤1	>16	≤1 - >16	50.9	13.2	35.9
	TZP	2	128	0.25 - >128	71.1	6.3	22.6
	MEM	≤0.06	0.25	≤0.06 - 4	99.4	0.6	0.0
	LVX	1	>8	0.015 - > 8	52.8	1.9	45.3
	AMK	4	16	1 - >64	86.8	6.3	6.9
Portugal (355)	TGC	0.5	1	≤0.008 - 8	90.4	5.9	3.7
	CAZ	≤1	>16	≤1 - >16	54.4	17.2	28.5
	TZP	2	128	0.25 - >128	72.1	9.3	18.6
	MEM	≤0.06	0.25	≤0.06 - >16	99.2	0.0	0.8
	LVX	0.12	> 8	≤0.008 - >8	71.0	1.7	27.3
	AMK	2	8	≤0.5 - 32	98.0	1.7	0.3
Romania (158)	TGC	0.5	1	0.06 - 4	90.5	7.0	2.5
	CAZ	≤1	>16	≤1 - >16	61.4	8.9	29.8
	TZP	2	>128	0.5 - >128	71.5	4.4	24.1
	MEM	≤0.06	2	≤0.06 - >16	90.5	2.5	7.0
	LVX	0.25	>8	0.015 - >8	60.8	4.4	34.8
	AMK	2	8	≤0.5 - >64	95.6	1.3	3.1
Spain (1,380)	TGC	0.25	1	0.015 - 8	93.1	4.9	2.0
	CAZ	≤1	16	≤1 - >16	71.1	12.7	16.2
	TZP	2	64	≤0.06 - >128	84.1	3.1	12.8
	MEM	≤0.06	0.25	≤0.06 - >16	99.0	0.7	0.3
	LVX	0.06	8	≤0.008 - >8	77.3	2.2	20.5
	AMK	2	4	≤0.5 - >64	99.1	0.4	0.5
Sweden (167)	TGC	0.25	1	0.06 - 4	95.8	2.4	1.8
	CAZ	≤1	8	≤1 - >16	86.2	3.6	10.2
	TZP	2	8	0.5 - >128	90.4	1.8	7.8
	MEM	≤0.06	0.12	≤0.06 - 1	100	0.0	0.0
	LVX	0.03	0.5	≤0.008 - >8	95.2	1.2	3.6
	AMK	2	4	1 - 16	99.4	0.6	0.0
Switzerland (85)	TGC	0.25	0.5	0.06 - 1	100	0.0	0.0
	CAZ	≤1	2	≤1 - >16	89.4	2.4	8.2
	TZP	1	64	0.25 - >128	85.9	3.5	10.6
	MEM	≤0.06	≤0.06	≤0.06 - 0.5	100	0.0	0.0
	LVX	0.06	0.5	0.015 - > 8	96.5	0.0	3.5
	AMK	2	4	≤0.5 - 16	98.8	1.2	0.0
United Kingdom (167)	TGC	0.5	1	0.06 - 8	95.8	2.4	1.8
	CAZ	≤1	16	≤1 - >16	83.2	4.2	12.6
	TZP	2	32	0.25 - >128	87.4	1.2	11.4
	MEM	≤0.06	0.12	≤0.06 - 8	99.4	0.6	0.0
	LVX	0.06	0.5	0.015 - >8	92.8	1.8	5.4
	AMK	2	4	≤0.5 - >64	98.2	1.2	0.6

TGC, tigecycline; CAZ, ceftazidime; TZP, piperacillin-tazobactam; MEM, meropenem; LVX, levofloxacin; AMK, amikacin. Breakpoints were interpreted according to EUCAST 2015 criteria. Percent susceptible ≥ 90% is shaded in purple.

Figure 1. Distribution of β-lactamases in molecularly characterized *Enterobacteriaceae* isolates, by country.



Countries: BEL, Belgium; HRV, Croatia; CZE, Czech Republic; DNK, Denmark; FIN, Finland; FRA, France; DEU, Germany; GRC, Greece; HUN, Hungary; IRL, Ireland; ITA, Italy; NLD, Netherlands; POL, Poland; PRT, Portugal; ROU, Romania; ESP, Spain; SWE, Sweden; CHE, Switzerland; GBR, United Kingdom.

Table 2. *In vitro* activity of tigecycline and comparator agents against subsets of *Enterobacteriaceae* isolates with select non-susceptible phenotypes.

Phenotype (no. of isolates)	Antimicrobial agent	MIC (mg/L) MIC_{50}	MIC_{90}	Range	MIC Interpretation (%) Susceptible	Intermediate	Resistant
CAZ-NS (2,276)	TGC	0.5	2	0.015 - >8	85.6	9.1	5.3
	CAZ	16	>16	2 - >16	0.0	27.8	72.2
	FEP	4	>32	≤0.5 - >32	34.4	17.5	48.1
	TZP	16	>128	≤0.06 - >128	47.3	11.3	41.4
	MEM	0.12	1	≤0.06 - >16	93.5	1.6	4.92
	LVX	1	>8	≤0.008 - >8	52.1	3.8	44.1
	AMK	2	8	≤0.5 - >64	91.7	5.1	3.3
MEM-NS (154)	TGC	1	2	0.06 - 8	69.5	22.1	8.4
	CAZ	>16	>16	≤1 - >16	2.6	0.0	97.4
	FEP	>32	>32	≤0.5 - >32	2.6	2.0	95.4
	TZP	>128	>128	4 - >128	1.3	0.0	98.7
	MEM	>16	>16	4 - >16	0.0	26.0	74.0
	LVX	>8	>8	0.03 - >8	6.5	3.9	89.6
	AMK	16	32	≤0.5 - >64	47.4	35.7	16.9
TZP-NS (1,503)	TGC	0.5	2	≤0.008 - >8	81.6	11.4	6.9
	CAZ	>16	>16	≤1 - >16	19.1	8.7	72.2
	FEP	4	>32	≤0.5 - >32	37.5	20.7	41.8
	TZP	64	>128	16 - >128	0.0	22.0	78.0
	MEM	0.12	4	≤0.06 - >16	89.9	2.6	7.5
	LVX	2	>8	≤0.008 - >8	49.9	4.1	46.0
	AMK	2	16	≤0.5 - >64	88.4	7.3	4.3
AMK-NS (219)	TGC	1	2	0.015 - 8	70.3	21.9	7.8
	CAZ	>16	>16	≤1 - >16	10.4	7.1	82.5
	FEP	>32	>32	≤0.5 - >32	15.1	9.1	75.8
	TZP	128	>128	0.5 - >128	20.1	7.8	72.1
	MEM	0.5	>16	≤0.06 - >16	63.0	3.7	33.3
	LVX	>8	>8	≤0.008 - >8	21.9	4.6	73.5
	AMK	16	>64	16 - >64	0.0	62.6	37.4

TGC, tigecycline; CAZ, ceftazidime; FEP, cefepime; TZP, piperacillin-tazobactam; MEM, meropenem; LVX, levofloxacin; AMK, amikacin; NS, non-susceptible. Breakpoints were interpreted according to EUCAST 2015 criteria.

Table 3. Cumulative MIC distributions for tigecycline against molecularly characterized *Enterobacteriaceae* isolates carrying β-lactamases.

	No. of isolates/ (cumulative % of isolates) inhibited at MIC (mg/L) of:[b]							
β-lactamase content (no.)[a]	**≤0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**≥8**
CTX-M-15 (556)	18 (3.2)	106 (22.3)	122 (44.2)	147 (70.7)	88 (86.5)	48 (**95.1**)	23 (99.3)	4 (100)
CTX-M-14 (65)	7 (10.8)	25 (49.2)	20 (80.0)	9 (**93.8**)	2 (96.9)	1 (98.5)	1 (100)	
SHV-ESBL (50)	2 (4.0)	11 (26.0)	9 (44.0)	15 (74.0)	6 (86.0)	4 (**94.0**)	3 (100)	
Other ESBL (137)[c]	13 (9.5)	51 (46.7)	33 (70.8)	21 (86.1)	10 (**93.4**)	6 (97.8)	3 (100)	
AmpC (14)	1 (7.1)	1 (14.3)	1 (21.4)	5 (57.1)	4 (85.7)	0 (85.7)	2 (**100**)	
AmpC + ESBL (7)		1 (14.3)	1 (28.6)	2 (57.1)	0 (57.1)	0 (57.1)	3 (100)	
KPC-2 ± ESBL ± AmpC (65)			8 (12.3)	14 (33.8)	27 (75.4)	11 (**92.3**)	4 (98.5)	1 (100)
KPC-3 ± ESBL ± AmpC (135)		3 (2.2)	6 (6.7)	29 (28.1)	59 (71.9)	29 (**93.3**)	6 (97.8)	3 (100)
OXA-48-like ± ESBL ± AmpC (59)[d]	1 (1.7)	0 (1.7)	15 (27.1)	17 (55.9)	11 (74.6)	9 (89.8)	6 (**100**)	
GES-5 + AmpC (2)			2 (100)					
NDM-1 ± ESBL ± AmpC (9)			1 (11.1)	5 (66.7)	1 (77.8)	2 (100)		
VIM ± ESBL ± AmpC (35)[e]		3 (8.6)	11 (40.0)	8 (62.9)	10 (**91.4**)	2 (97.1)	1 (100)	

[a]Includes isolates that also carry original spectrum β-lactamases (e.g. TEM-1, SHV-1, SHV-11).
[b]Dashed lines indicate susceptible breakpoint for tigecycline using EUCAST interpretive criteria (≤ 1mg/L, red) and U.S. FDA interpretive criteria (≤ 2 mg/L, blue). The MIC inhibiting 90% of isolates (MIC_{90}) is bolded. MIC_{90} was not calculated for n <10.
[c]Includes isolates carrying multiple ESBLs.[d]Includes isolates carrying OXA-48 (n=57), OXA-181 (n=1) and OXA-232 (n=1). [e]Includes isolates carrying VIM-1 (n=33), VIM-4 (n=1) and VIM-19 (n=1). Three isolates carried VIM-1 and KPC-2 and one isolate carried VIM-1 and OXA-48.

Results Summary

- 92.3% of the overall population of *Enterobacteriaceae* isolates collected in Europe in 2014 as part of TEST was susceptible *in vitro* to tigecycline, with ≤90% of isolates collected in 17 of 19 countries testing with MIC values ≤1 mg/L.
- Tigecycline showed comparable activity *in vitro* to that of meropenem and amikacin against ceftazidime-non-susceptible and piperacillin-tazobactam-non-susceptible isolates. Tigecycline was more active than other tested agents, including amikacin, against the subset of meropenem-non-susceptible isolates.
- 70% of amikacin-non-susceptible isolates were inhibited by ≤1 mg/L of tigecycline.
- Tigecycline was active against isolates carrying β-lactamases, with <85% of isolates carrying ESBLs (CTX-M-15, CTX-M-14, SHV-type, TEM-type, other CTX-M-type, and combinations thereof) susceptible at the EUCAST breakpoint and ≤94% of these isolates susceptible at the U.S. FDA breakpoint (≤2 mg/L).
- Similarly, tigecycline was active against <71% of isolates carrying serine carbapenemases (KPC, OXA-48-like, GES) or metallo-β-lactamases (NDM, VIM) when using EUCAST interpretive criteria. This compares to ≤90% susceptible at the FDA breakpoint.

Conclusions

- Tigecycline had good *in vitro* activity against *Enterobacteriaceae* that carried one or more β-lactamases, including combinations of ESBLs and carbapenemases.
- Tigecycline is one of the few currently available antimicrobial agents with significant activity against difficult-to-treat *Enterobacteriaceae* such as those producing VIM and NDM MBLs.

References and Acknowledgments:

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