Carbapenemases Found in Meropenem Non-Susceptible *Enterobacteriaceae* Collected as Part of the Tigecycline Evaluation Surveillance Trial (TEST) Program in 2011-2014.

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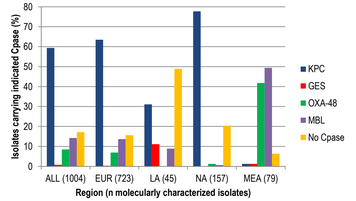
Revised Abstract

Objectives: TEST monitors the *in vitro* activity of tigecycline and other antimicrobials against clinically-relevant pathogens collected globally. This study reports the β-lactamase content of a subset of meropenem non-susceptible (MEM-NS) TEST isolates collected in Europe (EUR), Latin America (LA), and North America (NA) in 2011-2014 and Middle East/ Africa (MEA) in 2011-2013. **Methods**: Non-duplicate clinical isolates were collected from defined infection sites. Susceptibility testing was performed by broth microdilution by the local laboratory using supplied panels and interpreted using CLSI breakpoints. MEM-NS phenotype (MIC > 1 mg/L) was confirmed at IHMA before screening isolates for β-lactamase genes encoding carbapenemases (Cpase), extended-spectrum β-lactamases (ESBL) and AmpC β-lactamases. **Results**: 1,457 MEM-NS *Enterobacteriaceae* (of 52,079 total) were collected globally. The β-lactamase content of a subset of 1,004 MEM-NS isolates collected in EUR (723), LA (45), NA (157), and MEA (79) was determined. The antimicrobial susceptibilities and Cpase content of these isolates are shown:

	% Susceptible; MIC_{90}[1]			
Region (n)	TGC	TZP	LVX	AMK
All (52,079)	97.3; 1	85.0; 64	79.5; > 8	98.1; 4
All MEM-NS (1457)	91.6; 2	10.6; > 128	20.0; > 8	70.6; 64
Characterized MEM-NS (1004)	91.5; 2	4.1; > 128	14.8; > 8	74.4; 32
EUR (723)	92.3; 2	2.8; > 128	12.4; > 8	73.0; 32
LA (45)	95.6; 2	8.9; > 128	20.0; > 8	75.6; > 64
NA (157)	93.6; 2	10.2; > 128	20.4; > 8	87.9; 32
MEA (79)	78.5, 4	1.3; > 128	22.8; > 8	59.5; > 64

[1]TGC, tigecycline; TZP, piperacillin-tazobactam; LVX, levofloxacin; AMK, amikacin.



No Cpase, no plasmid-encoded Cpase detected by PCR. Includes isolates carrying ESBLs, AmpC and chromosomally-encoded β-lactamases, presumably in combination with changes in membrane permeability.

Conclusions: Regional differences in the incidence of Cpases and in the antimicrobial susceptibility (% S) of characterized MEM-NS isolates were observed. TGC remained active *in vitro* against > 92% of MEM-NS *Enterobacteriaceae* collected in EUR, LA, and NA and against >78.5% of isolates collected in MEA that carried a proportionately greater number of MBLs, and was more potent than other tested agents. TGC continues to display significant *in vitro* activity against difficult-to-treat *Enterobacteriaceae*.

Introduction

TEST monitors the *in vitro* activity of tigecycline and other antimicrobials against clinically-relevant pathogens collected globally. This study reports the β-lactamase content of a subset of meropenem non-susceptible (MEM-NS) TEST isolates collected in Europe (EUR), Latin America (LA), and North America (NA) in 2011-2014 and Middle East/ Africa (MEA) in 2011-2013.

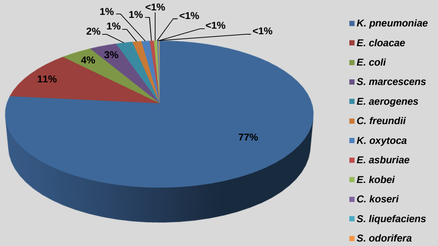
Materials & Methods

- Non-duplicate clinical isolates were collected from defined infection sites.
- Susceptibility testing was performed by broth microdilution by the local laboratory using supplied panels and interpreted using CLSI breakpoints [1,2]. Tigecycline data was interpreted using FDA breakpoints [3].
- MEM-NS phenotype (MIC > 1 mg/L) was confirmed at IHMA before screening isolates for β-lactamase genes encoding carbapenemases (Cpase; KPC, OXA-48, NDM, IMP, VIM, SPM), extended-spectrum β-lactamases (ESBL; SHV, TEM, CTX-M, VEB, PER, GES) and AmpC β-lactamases (ACC, ACT, CMY, DHA, MIR, MOX, FOX) by PCR, followed by sequencing.

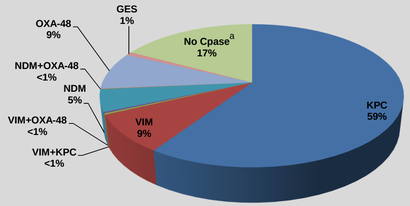
Results

Figure 1a-1f. Species distribution and β-lactamase content of molecularly characterized meropenem-non-susceptible *Enterobacteriaceae* isolates collected as part of TEST (2011-2014).

1a. Species composition of molecularly characterized meropenem-NS *Enterobacteriaceae* (n=1,004)

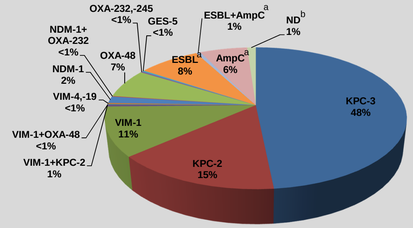


1b. β-lactamase content of all molecularly characterized meropenem-NS isolates (n=1,004)



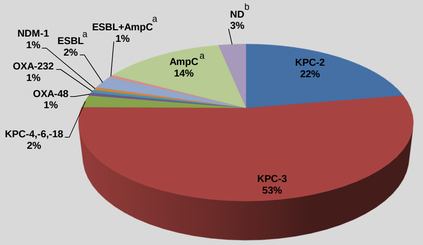
[a]No Cpase: no gene encoding a carbapenemase was detected. 97.7% of these isolates carried an extended spectrum β-lactamase (ESBL) or plasmid- or chromosomally-mediated AmpC β-lactamase presumably in combination with changes in membrane permeability

1c. β-lactamase content of molecularly characterized meropenem-NS isolates collected in Europe (n=723)



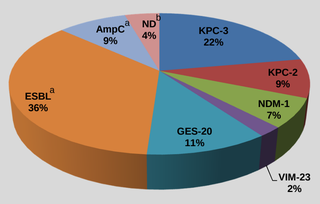
[a]Isolates carrying extended spectrum β-lactamases (ESBLs) or plasmid- or chromosomally-mediated AmpC β-lactamases are presumed to also harbor changes in membrane permeability
[b]ND: no β-lactamase identified

1d. β-lactamase content of molecularly characterized meropenem-NS isolates collected in North America (n=157)



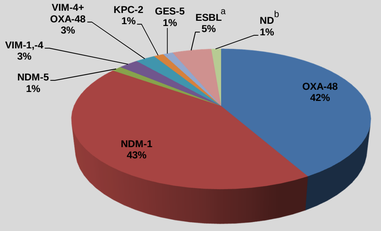
[a]Isolates carrying extended spectrum β-lactamases (ESBLs) or plasmid- or chromosomally-mediated AmpC β-lactamases are presumed to also harbor changes in membrane permeability
[b]ND: no β-lactamase identified

1e. β-lactamase content of molecularly characterized meropenem-NS isolates collected in Latin America (n=45)



[a]Isolates carrying extended spectrum β-lactamases (ESBLs) or plasmid- or chromosomally-mediated AmpC β-lactamases are presumed to also harbor changes in membrane permeability
[b]ND: no β-lactamase identified

1f. β-lactamase content of molecularly characterized meropenem-NS isolates collected in Middle East/Africa (n=79)



[a]Isolates carrying extended spectrum β-lactamases (ESBLs) or plasmid- or chromosomally-mediated AmpC β-lactamases are presumed to also harbor changes in membrane permeability
[b]ND: no β-lactamase identified

Table 1. *In vitro* activity of tigecycline and comparator agents tested against molecularly characterized meropenem non-susceptible *Enterobacteriaceae* isolates collected as part of the TEST program, 2011-2014

	MIC (μg/mL) [a]					
Group (n)	TGC	FEP	MEM	TZP	AMK	LVX
All *Enterobacteriaceae* (52,079)						
MIC_{50}/ MIC_{90}	0.5/ 1	≤0.5/ 16	≤0.06/ 0.25	2/ 64	2/ 4	0.06/ >8
Range	≤0.008-16	≤0.5->32	≤0.06->16	≤0.06->128	≤0.5->64	≤0.008->8
%Susceptible	97.3	81.9	97.2	85.0	98.1	79.5
All Meropenem-NS *Enterobacteriaceae* (1,457)						
MIC_{50}/ MIC_{90}	1/ 2	>32/ >32	16/ >16	>128/ >128	16/ 64	>8/ >8
Range	≤0.008-16	≤0.5->32	2->16	0.12->128	≤0.5->64	0.015->8
% Susceptible	91.6	7.6	0	10.6	70.6	20.0
Characterized Meropenem-NS *Enterobacteriaceae* (1,004)						
MIC_{50}/ MIC_{90}	1/ 2	>32/ >32	>16/ >16	>128/ >128	16/ 32	>8/ >8
Range	0.06->8	≤0.5->32	2->16	0.25->128	≤0.5->64	0.015->8
% Susceptible	91.5	3.6	0	4.1	74.4	14.8
Europe						
All *Enterobacteriaceae* (32,466)						
MIC_{50}/ MIC_{90}	0.5/ 1	≤0.5/ 32	≤0.06/ 0.25	2/ 64	2/ 4	0.06/ >8
Range	≤0.008-16	≤0.5->32	≤0.06->16	≤0.06->128	≤0.5->64	≤0.008->8
% Susceptible	97.5	80.8	97.1	83.8	98.2	79.4
Characterized Meropenem-NS (723)						
MIC_{50}/ MIC_{90}	1/ 2	>32/ >32	>16/ >16	>128/ >128	16/ 32	>8/ >8
Range	0.06->8	≤0.5->32	2->16	0.25->128	≤0.5->64	0.015->8
% Susceptible	92.3	1.8	0	2.8	73.0	12.4
KPC-positive (459)						
MIC_{50}/ MIC_{90}	1/ 2	>32/ >32	>16/ >16	>128/ >128	16/ 32	>8/ >8
Range	0.12->8	4->32	2->16	64->128	≤0.5->64	0.03->8
% Susceptible	93.9	0	0	0	65.4	4.1
MBL-positive (99)						
MIC_{50}/ MIC_{90}	1/ 2	>32/ >32	8/ >16	>128/ >128	4/ 16	>8/ >8
Range	0.06-8	4->32	2->16	64->128	1->64	0.015->8
% Susceptible	91.9	0	0	0	90.9	27.3
OXA-48 positive (50)						
MIC_{50}/ MIC_{90}	1/ 4	>32/ >32	4/ >16	>128/ >128	4/ 8	>8/ >8
Range	0.06-4	≤0.5->32	2->16	128->128	1->64	0.03->8
% Susceptible	86.0	10.0	0	0	92.0	14.0
GES positive (2)						
Range	0.25-0.25	16-16	>16->16	>128->128	2-4	2-2
% Susceptible	100	0	0	0	100	100
No Cpase (113)						
MIC_{50}/ MIC_{90}	1/ 4	>32/ >32	4/ >16	>128/ >128	4/ >64	>8/ >8
Range	0.06-8	≤0.5->32	2->16	0.25->128	≤0.5->64	0.03->8
% Susceptible	88.5	7.1	0	17.7	79.6	31.0
North America						
All *Enterobacteriaceae* (13,913)						
MIC_{50}/ MIC_{90}	0.5/ 1	≤0.5/ 2	≤0.06/ 0.12	2/ 16	2/ 4	0.06/ 8
Range	0.008->8	≤0.5->32	≤0.06->16	≤0.06->128	≤0.5->64	≤0.008->8
% Susceptible	97.3	90.4	98.2	90.8	99.3	84.6
Characterized Meropenem-NS (157)						
MIC_{50}/ MIC_{90}	1/ 2	>32/ >32	16/ >16	>128/ >128	4/ 32	>8/ >8
Range	0.06->8	≤0.5->32	2->16	0.5->128	≤0.5->64	0.03->8
% Susceptible	93.6	11.5	0	10.2	87.9	20.4
KPC-positive (122)						
MIC_{50}/ MIC_{90}	1/ 2	>32/ >32	16/ >16	>128/ >128	8/ 32	>8/ >8
Range	0.06-8	2->32	2->16	32->128	1->64	0.03->8
% Susceptible	92.6	0.8	0	0	86.9	6.6
MBL-positive (1)						
Range	1	>32	>16	>128	4	>8
% Susceptible	100	0	0	0	100	0
OXA-48-like positive (2)						
Range	0.12-4	>32->32	8-16	>128->128	1-16	8->8
% Susceptible	50.0	0	0	0	100	0
No Cpase (32)						
MIC_{50}/ MIC_{90}	0.5/ 2	2/ >32	8/ >16	16/ >128	2/ 16	0.25/ >8
Range	0.12-2	≤0.5->32	2->16	0.5->128	≤0.5-64	0.03->8
% Susceptible	100	53.1	0	50.0	90.6	75.0

	MIC (μg/mL) [a]					
Group (n)	TGC	FEP	MEM	TZP	AMK	LVX
Latin America						
All *Enterobacteriaceae* (3,216)						
MIC_{50}/ MIC_{90}	0.5/ 2	≤0.5/ >32	≤0.06/ 0.25	4/ >128	2/ 16	0.5/ >8
Range	≤0.008->8	≤0.5->32	≤0.06->16	0.12->128	≤0.5->64	≤0.008->8
% Susceptible	96.5	68.3	95.9	77.1	93.1	63.9
Characterized Meropenem-NS (45)						
MIC_{50}/ MIC_{90}	0.5/ 2	>32/ >32	8/ >16	>128/ >128	8/ >64	>8/ >8
Range	0.12-4	2->32	2->16	4->128	1->64	0.03->8
% Susceptible	95.6	2.2	0	8.9	75.6	20.0
KPC-positive (14)						
MIC_{50}/ MIC_{90}	0.5/ 2	>32/ >32	16/ >16	>128/ >128	16/ 32	>8/ >8
Range	0.12-2	8->32	2->16	32->128	1->64	0.03->8
% Susceptible	100	0	0	0	85.7	21.4
MBL-positive (4)						
Range	0.12-1	16->32	2-16	128->128	2-32	0.5->8
% Susceptible	100	0	0	0	50.0	75.0
GES-positive (5)						
Range	0.25-2	2->32	8->16	32->128	8->64	>8->8
% Susceptible	100	20.0	0	0	40.0	0
No Cpase (22)						
MIC_{50}/ MIC_{90}	0.25/ 1	>32/ >32	4/ >16	>128/ >128	8/ 32	>8/ >8
Range	0.12-4	4->32	2->16	4->128	1->64	0.5->8
% Susceptible	90.9	0	0	18.2	81.8	13.6
Middle East/Africa						
All *Enterobacteriaceae* (2,484)						
MIC_{50}/ MIC_{90}	0.5/ 2	≤0.5/ >32	≤0.06/ 0.5	2/ 128	2/ 8	0.25/ >8
Range	≤0.008-16	≤0.5->32	≤0.06->16	≤0.06->128	≤0.5->64	≤0.008->8
% Susceptible	96.4	64.8	94.4	79.6	95.9	72.2
Characterized Meropenem-NS (79)						
MIC_{50}/ MIC_{90}	1/ 4	>32/ >32	16/ >16	>128/ >128	8/ >64	>8/ >8
Range	0.12->8	≤0.5->32	2->16	8->128	1->64	0.03->8
% Susceptible	78.5	5.1	0	1.3	59.5	22.8
KPC-positive (1)						
Range	0.5	2	8	64	4	1
% Susceptible	100	100	0	0	100	100
MBL-positive (39)						
MIC_{50}/ MIC_{90}	1/ 4	>32/ >32	16/ >16	>128/ >128	16/ >64	>8/ >8
Range	0.12-8	2->32	4->16	64->128	4->64	0.3->8
% Susceptible	82.1	2.6	0	0	51.3	15.4
OXA-48-positive (33)						
MIC_{50}/ MIC_{90}	1/ 4	>32/ >32	4/ >16	>128/ >128	8/ 64	8/ >8
Range	0.12->8	≤0.5->32	2->16	128->128	1-64	0.06->8
% Susceptible	78.8	3.0	0	0	63.6	30.3
GES-positive (1)						
Range	4	2	>16	8	8	8
% Susceptible	0	100	0	100	100	0
No Cpase (5)						
Range	0.5-8	32->32	2->16	128->128	1-64	0.5->8
% Susceptible	60.0	0	0	0	80.0	20.0

[a]TGC, tigecycline; FEP, cefepime; MEM, meropenem; TZP, pipericillin-tazobactam; AMK, amikacin; LVX, levofloxacin. %S ≥ 90 is highlighted.

Results Summary

- KPC was proportionately more common among molecularly characterized isolates from NA (77%), EUR (63%), and LA (31%). GES was most prevalent in LA (11%). OXA-48 or MBLs were found in >90% of isolates collected in MEA (42% and 50%, respectively).
- The largest proportion of meropenem-NS isolates that carried ESBLs and/ or AmpCs, presumably in combination with permeability defects, was found in LA (49%).
- Tigecycline was more active *in vitro* against molecularly characterized meropenem-NS isolates than all tested β-lactams and levofloxacin.
- Tigecycline was more active *in vitro* than amikacin against KPC-producing isolates from EUR, LA and NA, and against isolates in which no carbapenemase was detected. Tigecycline showed greater or comparable *in vitro* activity to amikacin against MBL-producing isolates from EUR, LA, NA, and MEA. Tigecycline was less active *in vitro* against OXA-48-producing isolates from EUR and NA.

Conclusions

- Regional differences in the incidence of Cpases were observed.
- Tigecycline remained active *in vitro* against > 92% of MEM-NS *Enterobacteriaceae* collected in EUR, LA, and NA and against >78% of isolates collected in MEA that carried a proportionately greater number of MBLs and OXA-48 carbapenemases, and was more potent than other tested agents.
- Tigecycline continues to display significant *in vitro* activity against difficult-to-treat *Enterobacteriaceae*.

References and Acknowledgments:

1. Clinical Laboratory Standards Institute. 2015. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standards -- Tenth Edition. CLSI document M07-A10 Wayne, PA.
2. Clinical and Laboratory Standards Institute. 2015. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. CLSI Document M100-S25. Wayne, PA.
3. Tygacil®, 2014. Tigecycline FDA prescribing information. Pfizer, Inc., Collegeville, PA.

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