P1268

Molecular Profiling of β-Lactam-Resistant *Enterobacteriaceae* Collected as Part of the Tigecycline European Surveillance Trial (TEST) in 2014-2015

IHMA, Inc.
2122 Palmer Drive
Schaumburg, IL 60173 USA
Phone: +1.847.303.5003
Fax: +1.847.303.5601
www.ihmainc.com

M.G. Wise[1], K.M. Kazmierczak[1], H. Leister-Tebbe[2], D. Sahm[1] [1]IHMA, Inc., Schaumburg IL, USA [2]Pfizer Inc., Collegeville PA, USA

Revised Abstract

Objectives: The TEST surveillance program monitors the *in vitro* activity of tigecycline and other antimicrobials against clinically-relevant pathogens collected worldwide. This study reports the β-lactamase content *Enterobacteriaceae* isolates collected in 19 European countries in 2014-2015.
Methods: Non-duplicate clinical isolates were collected from defined infection sites. Susceptibility testing was performed by CLSI broth microdilution by the local laboratory using supplied panels and interpreted using EUCAST breakpoints. Confirmation of meropenem non-susceptibility and extended-spectrum β-lactamase (ESBL) phenotype was performed at IHMA, Inc. A subset of isolates that exhibited meropenem MIC values ≥ 2 mg/L and/or were positive for ESBL activity were molecularly examined for genes encoding ESBLs, carbapenemases (KPC, NDM, VIM, IMP, OXA-48 group) and AmpC β-lactamases.
Results: Over the 2014-2015 study years, 15,273 *Enterobacteriaceae* were collected from Belgium (1075), Croatia (291), Czech Republic (169), Denmark (254), Finland (245), France (2276), Germany (2471), Greece (401), Hungary (85), Ireland (327), Italy (2790), Netherlands (319), Poland (159), Portugal (623), Romania (160), Spain (2867), Sweden (167), Switzerland (336) and the United Kingdom (258). The overall collection was 70.8% susceptible to ceftazidime, 78.5% susceptible to cefepime, 97.3% susceptible to meropenem, and 92.3% susceptible to tigecycline. A subset of 2069 isolates (1162 *Klebsiella* spp., 803 *E. coli,* 84 *Enterobacter* spp. and 20 other *Enterobacteriaceae*) were molecularly characterized and the β-lactamase content is shown below:

Enzyme groups	France (277)	Greece (62)	Ireland (67)	Italy (701)	Portugal (73)	Spain (342)	United Kingdom (19)	Eastern (183)[†]	Nordic (29)[‡]	Belgium/ Netherlands (130)	Germany/ Switzerland (186)
ESBL (n=1487)	254 [91.7%]	16 [25.8%]	62 [92.5%]	395 (56.3%)	62 (84.9%)	243 (71.0%)	16 [84.2%]	150 [82.0%]	27 [93.1%]	102 [78.4%]	160 [86.0%]
AmpC (n=20)	5 (1.8%)	1 (1.6%)		4 (0.6%)		3 (0.9%)	1 (5.3%)			5 (3.8%)	1 (0.5%)
ESBL + AmpC (n=17)	9 (3.2%)	1 (1.6%)		3 (0.4%)		1 (0.3%)		1 (0.5%)	1 (3.4%)		1 (0.5%)
GES-5 + AmpC (n=2)					2 (2.7%)						
KPC (n=211)		12 (19.4%)		190 (27.1%)	3 (4.1%)	1 (0.3%)		2 (1.1%)		3 (2.3%)	
KPC + ESBL/AmpC (n=85)		20 (32.2%)		60 (8.6%)	1 (1.4%)	2 (0.6%)		2 (1.1%)			
KPC + VIM + ESBL/AmpC (n=3)		3 (4.8%)									
NDM + ESBL/AmpC (n=11)	1 (0.3%)	4 (6.4%)		2 (0.3%)		1 (0.3%)		2 (1.1%)			1 (0.5%)
OXA-48 (n=4)						3 (0.9%)					1 (0.5%)
OXA-48 + ESBL/AmpC (n=109)	3 (1.1%)		2 (3.0%)	1 (0.1%)		65 (19.0%)		14 (7.6%)		12 (9.2%)	12 (6.4%)
OXA-48 + VIM + ESBL/AmpC (n=1)								1 (0.5%)			
VIM (n=10)		1 (1.6%)	1 (1.5%)			6 (1.8%)				2 (1.5%)	
VIM + ESBL/AmpC (n=51)		4 (6.4%)		35 (5.0%)	2 (2.7%)	5 (1.5%)		3 (1.6%)		1 (0.8%)	1 (0.5%)
No ESBL, AmpC or Cpase identified (n=58)[*]	5 (1.8%)		2 (3.0%)	11 (1.6%)	3 (4.1%)	12 (3.5%)	2 (10.5%)	8 (4.4%)	1 (3.4%)	5 (3.8%)	9 (4.8%)

[†]Eastern= Croatia (n=71), Czech Republic (n=26), Hungary (n=18), Poland (n=22), Romania (n=46)
[‡]Nordic= Denmark (n=5), Finland (n=17), Sweden (n=7)
[*]Stable derepression of chromosomally-encoded β-lactamases was not examined

Conclusions: Country to country differences in β-lactamase frequency were noted. KPC was proportionally more numerous in Greece and Italy compared to other countries, whereas OXA-48 was proportionally most abundant in Spain. No IMP metallo-β-lactamases were observed.

Introduction

The Tigecycline European Surveillance Trial (TEST) evaluates the activity of tigecycline and comparators against clinically relevant pathogens collected worldwide. This study reports on *Enterobacteriaceae* isolated in Europe in 2014-15. The subset of this collection that exhibited meropenem MIC values ≥ 2 mg/L and/or were confirmed as ESBL producers by combination clavulanic acid testing were molecularly characterized for β-lactamase content.

Materials & Methods

- Clinical isolates were collected from defined infection sites.
- Antibiotic susceptibility testing was performed by broth microdilution by local laboratories using supplied panels (TREK Diagnostic Systems, West Sussex, England). MICs were interpreted using EUCAST breakpoints [1]. Confirmation of meropenem MICs and ESBL phenotypes was performed at IHMA, Inc.
- The subset of organisms with meropenem MIC values ≥ 2 mg/L and/or positive for ESBL production using the CLSI disk diffusion confirmatory test were screened for genes encoding ESBLs (SHV, TEM, CTX-M, VEB, PER, GES), acquired 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 PCR [2].
- Genes encoding ESBLs and carbapenemases were sequenced to determine enzyme variants by comparison to curated databases at the NCBI (www.ncbi.nlm.nih.gov) and the Lahey Clinic (www.lahey.org/studies).

Results

Table 1. List of countries submitting isolates for TEST in 2014-2015.

Country or country group	No. collected	Total no. molecularly characterized	No. of isolates characterized with meropenem MIC values ≥2 mg/L
Spain	2867	342	39
Italy	2790	701	281
Germany	2471	182	11
France	2276	277	7
Belgium	1075	127	15
Portugal	623	73	9
Greece	401	62	42
Switzerland	336	4	0
Ireland	327	67	3
Netherlands	319	3	0
United Kingdom	258	19	2
Eastern			
Croatia	291	71	4
Czech Republic	169	26	0
Hungary	85	18	0
Poland	159	22	1
Romania	160	46	19
Nordic			
Denmark	254	5	0
Finland	245	17	0
Sweden	167	7	0
Total	15273	2069	433

Figure 1. Proportion of ESBL types (A) and carbapenemase types (B) observed among the total number of ESBL-harboring and carbapenamase-harboring isolates, respectively.

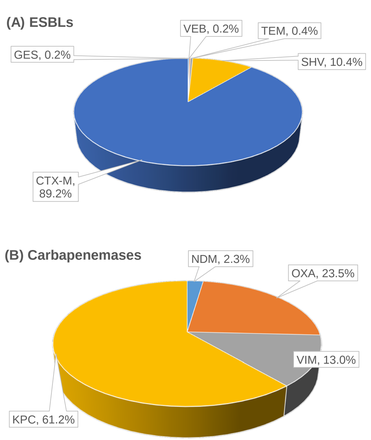


Table 2. Species harboring carbapenemases

Species (n)	KPC-2	KPC-3	KPC-2; VIM-1	NDM-1	OXA-181	OXA-232	OXA-48	OXA-48; VIM-1	VIM-1	VIM-19	VIM-4
Citrobacter freundii (n=9)	2	3					2		2		
Enterobacter aerogenes (n=4)		1		1			1		1		
Enterobacter cloacae (n=57)	1	1		7			8	1	38		1
Escherichia coli (n=8)		6			1				1		
Klebsiella oxytoca (n=6)							1		5		
Klebsiella pneumoniae (n=395)	77	205	3	2	2	3	92		10	1	
Serratia marcescens (n=6)				1			3		2		
Total	**80**	**216**	**3**	**11**	**3**	**3**	**107**	**1**	**59**	**1**	**1**

Figure 2. Proportion of individual ESBL enzyme variants found among the total number of ESBLs for each country grouping

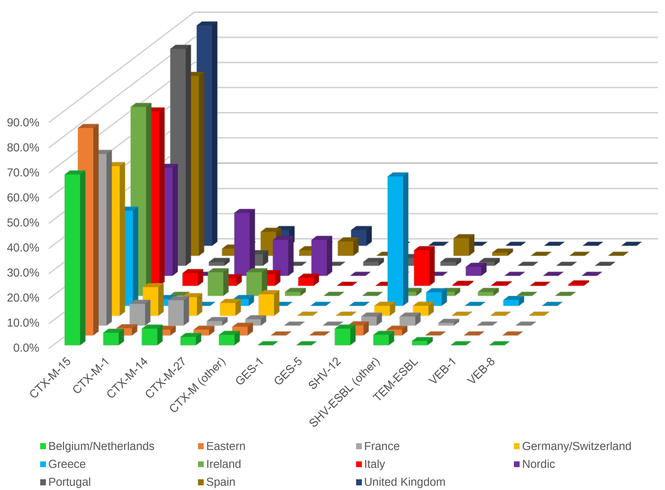
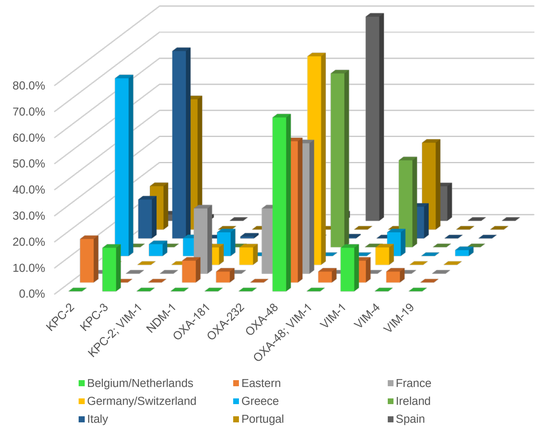


Table 3. *In vitro* activity of ceftazidime, meropenem and tigecycline against selected *Enterobacteriaceae* collected as part of TEST over 2014-2015 in Europe

Organism / phenotype/ genotype (n)	Drug	MIC (mg/L) MIC_{50}	MIC (mg/L) MIC_{90}	MIC (mg/L) MIC range	EUCAST MIC Interpretation Susceptible	Intermediate	Resistant
All *Enterobacteriaceae* (15,273)	CAZ	≤ 1	> 16	≤ 1 - >16	70.8	7.4	21.8
	MEM	≤ 0.06	0.12	≤ 0.06 - >16	97.2	0.70	2.1
	TGC	0.25	1	≤ 0.008 - >8	92.2	5.3	2.5
ESBL phenotype positive (1717)[1]	CAZ	16	>16	≤ 1 - >16	8.0	17.4	74.6
	MEM	≤0.06	2	≤0.06 - >16	92.0	2.2	5.8
	TGC	0.5	0.12	0.015 - >8	86.6	8.3	5.1
Meropenem non-susceptible (433)	CAZ	>16	>16	≤1 - >16	0.7	0	99.3
	MEM	>16	>16	4 - >16	0	23.6	76.4
	TGC	1	2	0.06 - >8	76.0	16.9	7.1
CTX-M-15 ±ESBL/AmpC (1227)	CAZ	16	>16	≤1 - >16	0.1	10.4	89.5
	MEM	≤0.06	2	≤0.06 - >16	91.4	3.2	5.4
	TGC	0.5	2	0.06 - >8	84.2	9.7	6.1
Serine Cpase ±ESBL/AmpC (413)	CAZ	>16	>16	≤ 1 - >16	0.7	0.5	98.8
	MEM	>16	>16	0.12 - >16	16.2	10.4	73.4
	TGC	1	2	0.06 - >8	75.3	17.7	7.0
MBL ±ESBL/AmpC (77)	CAZ	>16	>16	>16 - >16	0	0	100
	MEM	4	>16	2 - >16	27.3	48.0	24.7
	TGC	0.5	2	0.12 - >4	89.6	7.8	2.6

CAZ, ceftazidime; MEM, meropenem; TGC, tigecycline.
Cpase, carbapenemase; MBL, metallo-β-lactamase.
EUCAST Breakpoints (S|IR): CAZ (≤1 | 2-4 | ≥8); MEM (≤2 | 4-8 | ≥16); TGC (≤1 | 2 | ≥4).
[1]Phenotypically ESBL+: ESBL activity was assayed by the Kirby-Bauer disk diffusion method using cefotaxime, cefotaxime/clavulanate, ceftazidime, and ceftazidime/clavulanate antibiotic disks. An isolate was identified as ESBL+ if there was an increase of ≥5 mm in the inhibition zone diameter of the combination disk when compared to that of the cephalosporin alone. Only *E.coli, K. pneumoniae*, and *K. oxytoca* isolates were tested.

Figure 3. Proportion of individual carbapenemase enzyme variants found among the total number of carbapenemases for each country grouping



Conclusions

- The most common ESBL variant by far was CTX-M-15, followed by SHV-12 (which predominated in Greece), CTX-M-14 and CTX-M-1. VEB-1 was only found in Greece and VEB-8 only in Italy. GES variants were only found in Portugal.
- The most common carbapenemase detected was KPC-3, followed by OXA-48, KPC-2 and VIM-1. 85.6% of the characterized carbapenemase - producing isolates were from Italy, Spain and Greece. Most of these were KPC-positive isolates from Greece and Italy, but OXA-48-positive isolates dominated Spain. No *Enterobacteriaceae* carrying IMP metallo-β-lactamases were observed.
- TGC and MEM were more active than CAZ *in vitro* against the overall collection of *Enterobacteriaceae*, against phenotypically ESBL + isolates, and against characterized isolates producing CTX-M-15.
- TGC was more active than CAZ against the MEM-NS isolates. TGC was more active than CAZ or MEM against characterized isolates producing serine carbapenemases or MBLs.
- Tigecycline continues to exhibit strong antibacterial activity against *Enterobacteriaceae* that are difficult to treat with other available agents.

References

1. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 7.0, 2017. http://www.eucast.org.
2. Kazmierczak KM, Lob SH, Hoban DJ, Hackel MA, Badal RE, Bouchillon SK. 2015. Characterization of extended-spectrum β-lactamases and antimicrobial resistance of *Klebsiella pneumoniae* in intra-abdominal infection isolates in Latin America, 2008-2012. Results of the Study for Monitoring Antimicrobial Resistance Trends. Diagn Microbiol Infect Dis 82: 209-214.

Acknowledgments

We gratefully acknowledge the contribution of the investigators, laboratory personnel, and all members of the Tigecycline European Surveillance Trial group. This study was sponsored by Pfizer Inc. IHMA is a clinical research organization that has been contracted by Pfizer to manage the TEST program. DS, MW and KK are employees of IHMA, Inc., which was paid by Pfizer to manage this study and to prepare this poster. HL-T is an employee of Pfizer.