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Molecular profiling of beta-lactam-resistant Enterobacteriaceae collected as part of the tigecycline European surveillance trial (TEST) in 2014-2015

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Background: 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 of *Enterobacteriaceae* isolates collected in 19 European countries in 2014-2015. This information is important for understanding the challenges facing new antimicrobials in the future.

Material/methods: Non-duplicate clinical isolates were collected from defined specimen sources. Susceptibility testing was performed according to CLSI broth microdilution guidelines by the local laboratory using supplied panels and results were 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 were meropenem non-susceptible (MIC > 2 mg/L) and/or 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, 15,191 *Enterobacteriaceae* were collected from Belgium (1075), Croatia (291), Czech Republic (169), Denmark (254), Finland (245), France (2276), Germany (2471), Greece (319), 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)[a]	Nordic (29)[b]	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=84)		19 (30.6%)		60 (8.6%)	1 (1.4%)	2 (0.6%)		2 (1.1%)			
KPC + VIM + ESBL/AmpC (n=4)		4 (6.4%)									
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)[c]	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%)

[a]Eastern= Croatia (n=71), Czech Republic (n=26), Hungary (n=18), Poland (n=22), Romania (n=46)

[b]Nordic= Denmark (n=5), Finland (n=17), Sweden (n=7)

[c]Stable derepression of chromosomally-encoded β-lacatamases 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. IMP metallo-β-lactamases were not detected and they continue to be rare in Europe.