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Distribution of β-lactamases in Gram-negative pathogens from urinary tract infections in Europe – SMART 2010-2014

K. Kazmierczak[1], M. Hackel[1], V. DiLorenzo[2], S. Magnet[2], S. Lob[1], R. Badal[1], S. Bouchillon[1], D. Sahm[1]
[1]International Health Management Associates, Schaumburg, IL, USA
[2]IHMA Europe Sàrl, 9A Route de la Corniche, 1066 Epalinges, Switzerland

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

Revised Abstract

Background: The Study for Monitoring Antimicrobial Resistance Trends (SMART) tracks *in vitro* activity of antimicrobials used to treat intra-abdominal and urinary tract infections (UTI). In this analysis, we identified β-lactamases carried by *Enterobacteriaceae* collected from patients with UTI in European countries from 2010-2014. Methods: Fifty-seven laboratories in 18 countries each collected up to 50 consecutive, non-duplicate Gram-negative isolates from UTI per study year. Susceptibility and extended-spectrum β-lactamase (ESBL) phenotypes were determined using CLSI broth microdilution and interpreted using EUCAST 2015 breakpoints. Per SMART protocol, all ertapenem non-susceptible (ETP-NS; MIC > 0.5 mg/L) isolates and a randomly selected 50% of ESBL-phenotype positive (ESBLp+) *Escherichia coli, Klebsiella pneumoniae*, *K. oxytoca*, and *Proteus mirabilis* collected from each country are molecularly characterized for genes encoding ESBLs, carbapenemases (Cpases) and AmpC cephalosporinases. Results: 10,205 *Enterobacteriaceae* were collected from UTI in Europe in 2010-2014. Of these, 29.9% were non-susceptible to levofloxacin, the first-in-line empiric treatment for UTI, and 3.3% were ETP-NS. 1,382 isolates (692 *E. coli*, 567 *K. pneumoniae*, 60 *Enterobacter cloacae*, 30 *P. mirabilis*, and 33 other *Enterobacteriaceae*) were molecularly characterized. Serine- or metallo-carbapenemases were identified in 42%, 60%, 27%, and 100% of ETP-NS *E. coli*, *K. pneumoniae*, *E. cloacae* and *P. mirabilis* isolates, respectively. ESBLs were found in 95%, 86%, 30%, and 73% of all molecularly characterized isolates of these species

Phenotype/ Enzyme groups	*E. coli*	*K. pneumoniae*	*E. cloacae*	*P. mirabilis*
Total ESBLp+, ETP-S characterized	668	334	NA	27
ESBL	634	318		20
ESBL+AmpC	4	4		2
AmpC				3
Cpase+ESBL		1[a]		
No ESBL, AmpC, or Cpase detected[b]	30	11		2
Total ETP-NS characterized[c]	24	233	60	3
Cpase	2	59		
Cpase+ESBL	8	63		
Cpase+ESBL+AmpC		14	8	
Cpase+AmpC		4	8	3
ESBL	9	84		
ESBL+AmpC	1	5	10	
AmpC	1		34	
No ESBL, AmpC, or Cpase detected	3	4		
Total characterized	692	567	60	30
Total collected	6308	1760	335	777
% ESBLp+	16.6%	38.7%	NA	7.9%
% ETP-NS	0.4%	13.2%	17.9%	0.4%
% LVX-NS	31.4%	38.0%	22.4%	23.3%

ESBLp, ESBL phenotype; ETP, ertapenem; LVX, levofloxacin; S, susceptible; NS, non-susceptible; NA, not applicable.
[a]VIM-positive isolate that showed intermediate resistance to imipenem (MIC 8 mg/L)
[b]Includes SHV and TEM β-lactamases with undefined spectrum of activity
[c]Includes both ESBL phenotype-positive and phenotype-negative isolates

Conclusions: Carriage of ESBLs and Cpases differed between species of *Enterobacteriaceae* most commonly collected from patients with UTI in Europe. Though isolates producing one or more β-lactamases are increasingly common, ertapenem remained active (MIC ≤ 0.5 mg/L) *in vitro* against 96.7% of *Enterobacteriaceae* collected from UTI in Europe in 2010-2014.

Introduction

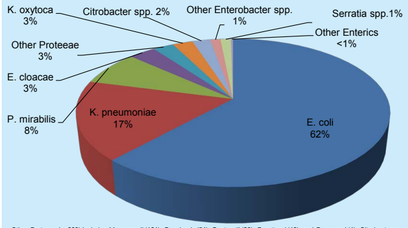
The Study for Monitoring Antimicrobial Resistance Trends (SMART) tracks *in vitro* activity of antimicrobials used to treat intra-abdominal and urinary tract infections (UTI). In this analysis, we identified β-lactamases carried by the four most common species of *Enterobacteriaceae* collected from patients with UTI in European countries from 2010-2014.

Materials & Methods

- Fifty-seven laboratories in 18 countries each collected up to 50 consecutive, non-duplicate Gram-negative isolates from UTI per study year.
- Susceptibility and extended-spectrum β-lactamase (ESBL) phenotypes were determined using CLSI broth microdilution [1, 2] and interpreted using EUCAST 2015 breakpoints [3].
- All ertapenem non-susceptible (ETP-NS; MIC > 0.5 mg/L) isolates and a randomly selected 50% of *Escherichia coli*, *Klebsiella pneumoniae*, *K. oxytoca*, and *Proteus mirabilis* isolates collected from each country that were ESBL-phenotype positive (ESBLp+) by combination clavulanic acid testing [2] were screened for the presence of β-lactamase genes encoding ESBLs (SHV, TEM, CTX-M, VEB, PER, GES), original-spectrum β-lactamases (e.g. TEM-1, SHV-1, SHV-11), carbapenemases (Cpases; KPC, OXA-48, NDM, VIM, IMP, SPM) and AmpC cephalosporinases (ACC, ACT, CMY, DHA, FOX, MIR, MOX) using PCR and microarray, followed by sequencing [4].

Results

Figure 1. Species distribution of *Enterobacteriaceae* isolates (n=10,205) collected from patients with UTI in European countries in 2010-2014.



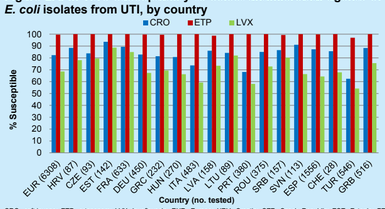
Other Proteae (n=260) includes *M. morganii* (161), *P. vulgaris* (54), *P. stuartii* (23), *P. rettgeri* (18), and *P. penneri* (4); *Citrobacter* spp. (n=224) includes *C. koseri* (112), *C. freundii* (99), *C. braakii* (8), *C. amalonaticus* (2), *C. murliniae* (1), *C. sedlakii* (1), and species undefined (1). Other *Enterobacter* spp. (n=130) includes *E. aerogenes* (113), *E. asburiae* (14), *E. kobei* (2), and *E. ludwigii* (1); *Serratia* spp. (n=130) includes *S. marcescens* (122), *S. liquefaciens* (4), *S. odorifera* (3), and *S. umslyitica* (1); Other Enterics (n=27) includes *Salmonella*, species undefined (8), *R. ornithinolytica* (7), *K. plantticola* (3), *P. agglomerans* (3), *H. alvei* (2), *K. cosenae* (2), *P. gergoviae* (1), and *Yersinia*, species undefined (1).

Table 1. *In vitro* activity of antimicrobial agents against *Enterobacteriaceae* from UTI collected in Europe in 2010-2014.

Organism (no. tested)	Antimicrobial agent	MIC (mg/L) MIC_{50}	MIC_{90}	Range	Interpretation[a] % Susceptible
Enterobacteriaceae, UTI (10,205)	Ceftriaxone	≤1	>32	≤1 - >32	76.4
	Ceftazidime	≤0.5	64	≤0.5 - >128	77.4
	Cefepime	≤0.5	>32	≤0.5 - >32	79.2
	Piperacillin-tazobactam	≤2	32	≤2 - >64	84.4
	Ertapenem	≤0.03	0.12	≤0.03 - >4	**96.7**
	Imipenem	0.12	1	≤0.06 - >8	**95.8**
	Levofloxacin	≤0.5	>4	≤0.5 - >4	70.1
	Amikacin	≤4	8	≤4 - >32	**93.4**
E. coli, UTI (6,308)	Ceftriaxone	≤1	>32	≤1 - >32	82.4
	Ceftazidime	≤0.5	16	≤0.5 - >128	83.5
	Cefepime	≤0.5	>32	≤0.5 - >32	83.8
	Piperacillin-tazobactam	≤2	16	≤2 - >64	89.9
	Ertapenem	≤0.03	≤0.03	≤0.03 - >4	**99.6**
	Imipenem	0.12	0.25	≤0.06 - >8	**99.9**
	Levofloxacin	≤0.5	>4	≤0.5 - >4	68.6
	Amikacin	≤4	8	≤4 - >32	**95.0**
K. pneumoniae, UTI (1,760)	Ceftriaxone	≤1	>32	≤1 - >32	56.6
	Ceftazidime	≤0.5	>128	≤0.5 - >128	56.6
	Cefepime	≤0.5	>32	≤0.5 - >32	57.7
	Piperacillin-tazobactam	4	>64	≤2 - >64	62.7
	Ertapenem	≤0.03	2	≤0.03 - >4	86.8
	Imipenem	0.25	1	≤0.06 - >8	**93.8**
	Levofloxacin	≤0.5	>4	≤0.5 - >4	62.0
	Amikacin	≤4	16	≤4 - >32	87.5
P. mirabilis, UTI (777)	Ceftriaxone	≤1	32	≤1 - >32	84.0
	Ceftazidime	≤0.5	8	≤0.5 - >128	84.9
	Cefepime	≤0.5	4	≤0.5 - >32	85.2
	Piperacillin-tazobactam	≤2	4	≤2 - >64	**95.7**
	Ertapenem	≤0.03	≤0.03	≤0.03 - >4	**99.6**
	Imipenem	2	4	≤0.06 - >8	72.1
	Levofloxacin	≤0.5	>4	≤0.5 - >4	76.7
	Amikacin	≤4	8	≤4 - >32	**90.5**
E. cloacae, UTI (335)	Ceftriaxone	≤1	>32	≤1 - >32	53.7
	Ceftazidime	1	>128	≤0.5 - >128	55.5
	Cefepime	≤0.5	>32	≤0.5 - >32	65.4
	Piperacillin-tazobactam	4	>64	≤2 - >64	65.4
	Ertapenem	≤0.03	2	≤0.03 - >4	82.1
	Imipenem	0.5	1	≤0.06 - >8	**95.2**
	Levofloxacin	≤0.5	> 4	≤0.5 - >4	77.6
	Amikacin	≤4	8	≤4 - >32	**92.8**

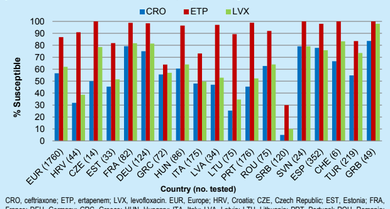
[a]% Susceptibility was determined using EUCAST 2015 interpretive criteria.

Figure 2A. *In vitro* susceptibility to select antimicrobial agents of *E. coli* isolates from UTI, by country



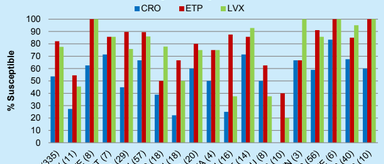
CRO, ceftriaxone; ETP, ertapenem; LVX, levofloxacin. EUR, Europe; HRV, Croatia; CZE, Czech Republic; EST, Estonia; FRA, France; DEU, Germany; GRC, Greece; HUN, Hungary; ITA, Italy; LVA, Latvia; LTU, Lithuania; PRT, Portugal; ROU, Romania; SRB, Serbia; SVN, Slovenia; ESP, Spain; CHE, Switzerland; TUR, Turkey; GBR, United Kingdom.

Figure 2B. *In vitro* susceptibility to select antimicrobial agents of *K. pneumoniae* isolates from UTI, by country



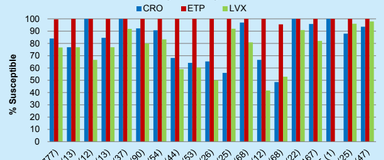
CRO, ceftriaxone; ETP, ertapenem; LVX, levofloxacin. EUR, Europe; HRV, Croatia; CZE, Czech Republic; EST, Estonia; FRA, France; DEU, Germany; GRC, Greece; HUN, Hungary; ITA, Italy; LVA, Latvia; LTU, Lithuania; PRT, Portugal; ROU, Romania; SRB, Serbia; SVN, Slovenia; ESP, Spain; CHE, Switzerland; TUR, Turkey; GBR, United Kingdom.

Figure 2C. *In vitro* susceptibility to select antimicrobial agents of *E. cloacae* isolates from UTI, by country



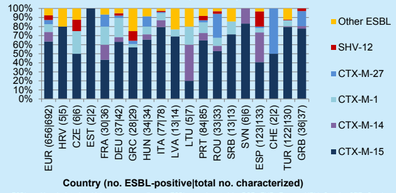
CRO, ceftriaxone; ETP, ertapenem; LVX, levofloxacin. EUR, Europe; HRV, Croatia; CZE, Czech Republic; EST, Estonia; FRA, France; DEU, Germany; GRC, Greece; HUN, Hungary; ITA, Italy; LVA, Latvia; LTU, Lithuania; PRT, Portugal; ROU, Romania; SRB, Serbia; SVN, Slovenia; ESP, Spain; CHE, Switzerland; TUR, Turkey; GBR, United Kingdom.

Figure 2D. *In vitro* susceptibility to select antimicrobial agents of *P. mirabilis* isolates from UTI, by country



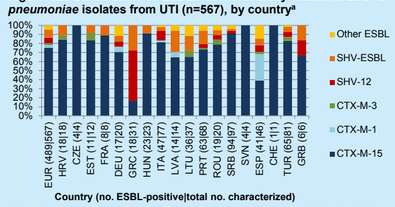
CRO, ceftriaxone; ETP, ertapenem; LVX, levofloxacin. EUR, Europe; HRV, Croatia; CZE, Czech Republic; EST, Estonia; FRA, France; DEU, Germany; GRC, Greece; HUN, Hungary; ITA, Italy; LVA, Latvia; LTU, Lithuania; PRT, Portugal; ROU, Romania; SRB, Serbia; SVN, Slovenia; ESP, Spain; CHE, Switzerland; TUR, Turkey; GBR, United Kingdom.

Figure 3A. Distribution of ESBLs in molecularly characterized *E. coli* isolates from UTI (n=692), by country[a]



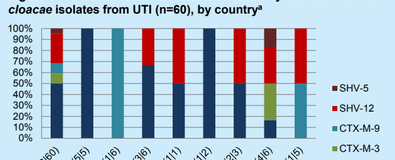
[a]ESBL-positive, isolate in which a gene encoding an ESBL was detected by PCR. EUR, Europe; HRV, Croatia; CZE, Czech Republic; EST, Estonia; FRA, France; DEU, Germany; GRC, Greece; HUN, Hungary; ITA, Italy; LVA, Latvia; LTU, Lithuania; PRT, Portugal; ROU, Romania; SRB, Serbia; SVN, Slovenia; ESP, Spain; CHE, Switzerland; TUR, Turkey; GBR, United Kingdom. Other ESBL, includes SHV-, TEM-, CTX-M-, and VEB-type enzymes.
Five isolates co-carried a CMY- or DHA-type AmpC.

Figure 3B. Distribution of ESBLs in molecularly characterized *K. pneumoniae* isolates from UTI (n=567), by country[a]



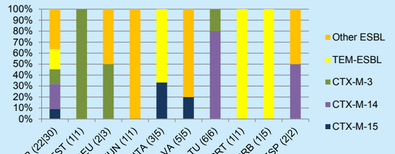
[a]ESBL-positive, isolate in which a gene encoding an ESBL was detected by PCR. EUR, Europe; HRV, Croatia; CZE, Czech Republic; EST, Estonia; FRA, France; DEU, Germany; GRC, Greece; HUN, Hungary; ITA, Italy; LVA, Latvia; LTU, Lithuania; PRT, Portugal; ROU, Romania; SRB, Serbia; SVN, Slovenia; ESP, Spain. Other ESBL, includes SHV-, TEM- and CTX-M-type enzymes.
Nineteen isolates co-carried a CMY- or DHA-type AmpC.

Figure 3C. Distribution of ESBLs in molecularly characterized *E. cloacae* isolates from UTI (n=60), by country[a]



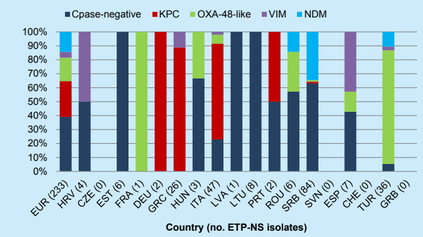
[a]ESBL-positive, isolate in which a gene encoding an ESBL was detected by PCR. EUR, Europe; HRV, Croatia; DEU, Germany; HUN, Hungary; LVA, Latvia; LTU, Lithuania; ROU, Romania; SRB, Serbia; ESP, Spain.

Figure 3D. Distribution of ESBLs in molecularly characterized *P. mirabilis* isolates from UTI (n=30), by country[a]



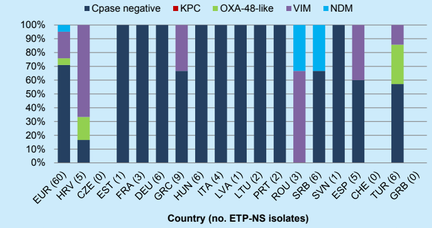
[a]ESBL-positive, isolate in which a gene encoding an ESBL was detected by PCR. EUR, Europe; EST, Estonia; DEU, Germany; HUN, Hungary; ITA, Italy; LVA, Latvia; LTU, Lithuania; PRT, Portugal; SRB, Serbia; ESP, Spain. Other ESBL, includes CTX-M-type enzymes.
Two isolates co-carried a CMY-type AmpC.

Figure 4A. Distribution of ETP-NS *K. pneumoniae* isolates (n=233) with different resistance mechanisms, by country[a].



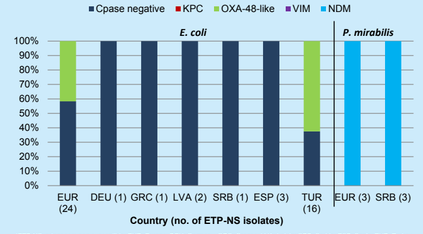
[a]ETP-NS, ertapenem non-susceptible. EUR, Europe; HRV, Croatia; CZE, Czech Republic; EST, Estonia; FRA, France; DEU, Germany; GRC, Greece; HUN, Hungary; ITA, Italy; LVA, Latvia; LTU, Lithuania; PRT, Portugal; ROU, Romania; SRB, Serbia; SVN, Slovenia; ESP, Spain; CHE, Switzerland; TUR, Turkey; GBR, United Kingdom.
Isolates carried genes for multiple carbapenemases (n=4): KPC-3 and OXA-48 (Italy); NDM-1 and OXA-48 (Romania); VIM-1, NDM-1 and OXA-244 (Turkey); VIM-1 and KPC-2 (Greece). Cpase-negative isolates (n=93): 88 isolates carried CTX-M-15 alone or with an SHV- or TEM-type ESBL or DHA-type AmpC; 1 carried CTX-M-3; only original spectrum β-lactamases were detected in 4 isolates. All isolates were presumed to harbor changes in outer membrane permeability.

Figure 4B. Distribution of ertapenem-non-susceptible *E. cloacae* isolates (n=60) with different resistance mechanisms, by country[a].



[a]ETP-NS, ertapenem non-susceptible. EUR, Europe; HRV, Croatia; CZE, Czech Republic; EST, Estonia; FRA, France; DEU, Germany; GRC, Greece; HUN, Hungary; ITA, Italy; LVA, Latvia; LTU, Lithuania; PRT, Portugal; ROU, Romania; SRB, Serbia; SVN, Slovenia; ESP, Spain; CHE, Switzerland; TUR, Turkey; GBR, United Kingdom.
Two isolates carried genes for multiple carbapenemases: VIM-1 and OXA-48 (Croatia); VIM-31 and OXA-48 (Turkey). Cpase-negative isolates (n=44): All isolates were presumed to carry the intrinsic ACT-type AmpC enzyme common to *E. cloacae* and to harbor changes in outer membrane permeability; 10 isolates also carried ESBLs (CTX-M-15, CTX-M-3, CTX-M-9, SHV-5, SHV-12).

Figure 4C. Distribution of ertapenem-non-susceptible *E. coli* (n=24) and *P. mirabilis* (n=3) isolates with different resistance mechanisms, by country [a].



[a]ETP-NS, ertapenem non-susceptible. EUR, Europe; DEU, Germany; GRC, Greece; LVA, Latvia; SRB, Serbia; ESP, Spain; TUR, Turkey. Cpase-negative isolates (n=14): 9 isolates carried CTX-M-15 alone or with CTX-M-14 or a CMY-type AmpC; 1 each carried CTX-M-1 or a CMY-type AmpC; only original spectrum β-lactamases were detected in 3 isolates. All isolates were presumed to harbor changes in outer membrane permeability.

Results Summary

- *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *E. cloacae* were the most prevalent species isolated from patients with UTI in Europe in 2010-2014.
- The four species displayed different antimicrobial susceptibility profiles to β-lactams and other class agents, with third-generation cephalosporins (ceftriaxone, ceftazidime) active *in vitro* against 84%, 82%, 56%, and 53% of *P. mirabilis*, *E. coli*, *K. pneumoniae*, and *E. cloacae* isolates, respectively.
- Ertapenem was active *in vitro* against >99% of *E. coli* and *P. mirabilis* isolates, 86% of *K. pneumoniae,* and 82% of *E. cloacae* isolates, whereas imipenem was active against >93% of *E. coli*, *K. pneumoniae*, and *E. cloacae* isolates. In comparison, levofloxacin activity ranged between 62-78% against these species.
- Antimicrobial susceptibility also differed between countries, with reduced susceptibility to different β-lactams correlated with carriage of ESBLs (predominantly CTX-M-15) and Cpases such as metallo-β-lactamases (e.g. VIM in Croatia and Greece, NDM in Serbia) and KPC in Italy and Greece.

Conclusions

- Carriage of ESBLs and Cpases differed between four species of *Enterobacteriaceae* most commonly collected from patients with UTI in Europe.
- Though isolates producing one or more β-lactamases are increasingly common, ertapenem remained active (MIC ≤ 0.5 mg/L) *in vitro* against 96.7% of overall *Enterobacteriaceae* collected from UTI in Europe in 2010-2014.
- Country-specific differences in β-lactamase prevalence should be taken into consideration when choosing empiric treatment.

References and Acknowledgments:

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