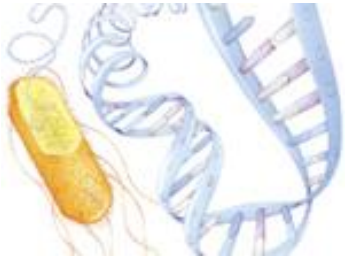


Resistance mechanisms to β-lactams among Gram-negative bacilli in clinical practice

TD Daniel Huang, MD, PhD

National Reference Centre for Antibiotic-Resistant Gram-Negative Bacilli
Laboratory of Clinical Microbiology, CHU UCL Namur, Mont-Godinne

SBIMC-BVIKM 2019-11-14

Burden of infections with AMR in EU

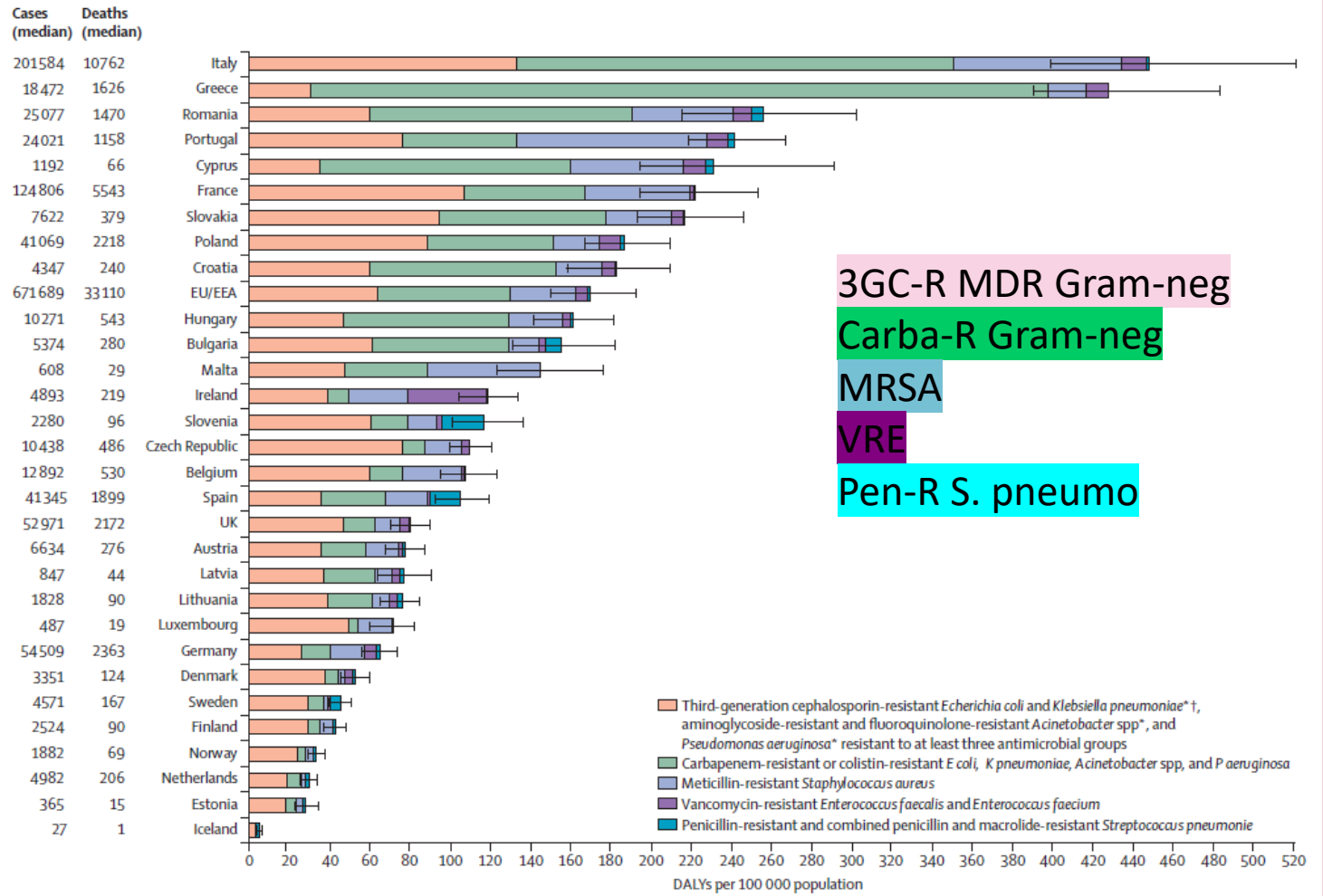


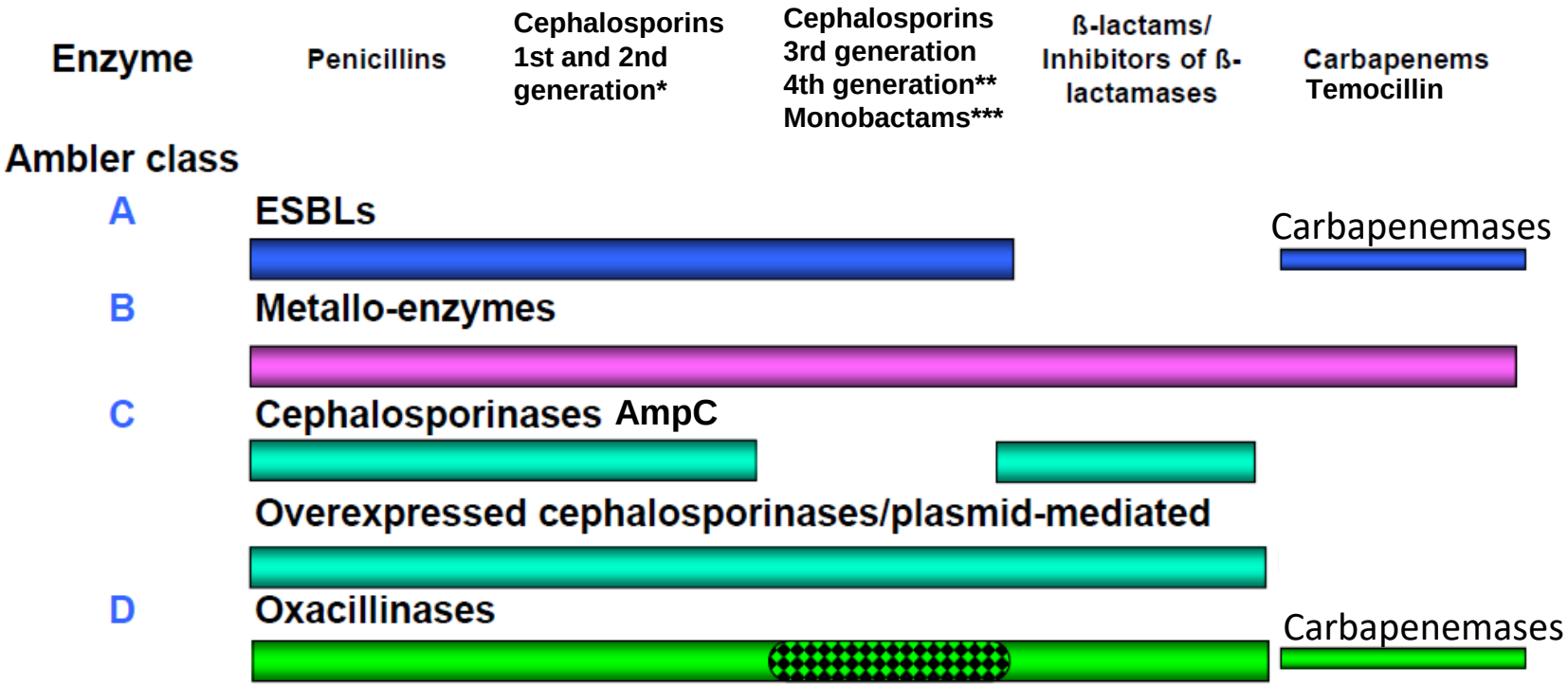
***Figure 3*: Burden of infections with antibiotic-resistant bacteria in DALYs, EU and European Economic Area, 2015**

Error bars are 95% uncertainty intervals. Greece did not report data on *S pneumoniae* isolates to the European Antimicrobial Resistance Surveillance Network in 2015. DALY rates are age-standardised to limit the effect of demographic differences across countries; numbers of cases and deaths are not age-standardised. DALYs=disability-adjusted life-years. *Excludes those resistant to carbapenem or colistin. †In 2015, most of the third-generation cephalosporin-resistant *E coli* (88·6%) and *K pneumoniae* (85·3%) isolates reported to the European Antimicrobial Resistance Surveillance Network produced an extended-spectrum β-lactamase.[9]

CHU UCL NAMUR

Cassini et al. LID 2018

Activity of large spectrum ß-lactamases



* Cephamycins excluded for ESBLs

** 4th generation cephalosporins excluded for overexpressed cephalosporinase

*** Monobactams (aztreonam) excluded for metallo-beta-lactamases

Epidemiology of ESBL-*Enterobacteriaceae*

- **Worldwide dissemination :**
  - **International pandemic CTX-M ESBL**
  - Vertical and horizontal transmission within and between species → Diffusion in all human medical sectors → **Difficult to control !**
- **Increasing resistance to 3GC (EARS-Net 2018*):**
  - 3GC-R E. coli : mean EU 14.9% (Belgium 10%)
  - 3GC-R K. pneumoniae : mean EU 31.2% (Belgium 19%)
- **Major threat for public health:**
  - Frequent among commensal flora: *E. coli* (70% among all ESBL species)
  - Most common pathogen of urinary tract infection in **community**
  - Multidrug-resistant (aminoglycosides, quinolones, cotrimoxazole,…) → major driver for **carbapenem use**

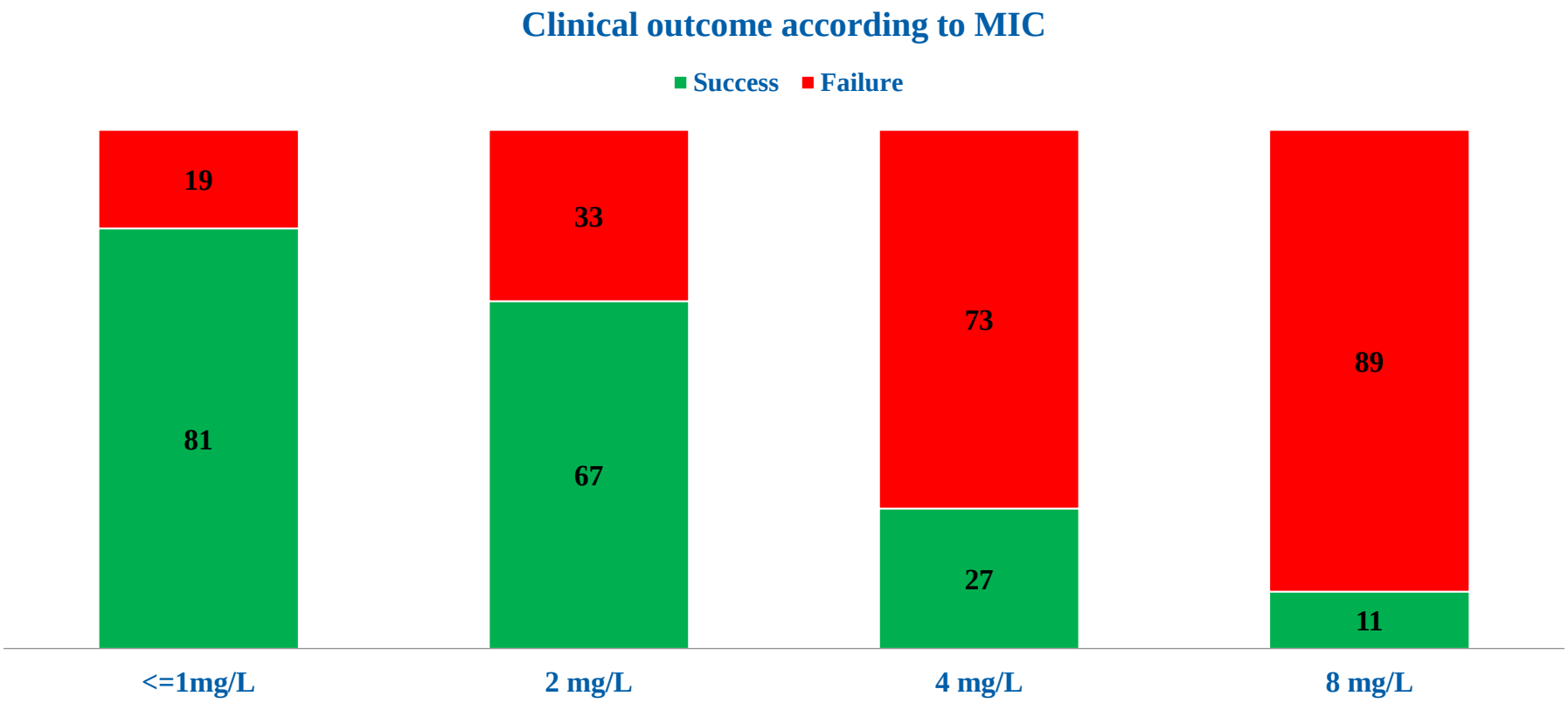
CHU UCL NAMUR

*Surveillance of antimicrobial resistance in Europe EARS-Net Report 2017, www.ecdc.europa.eu

Treatment of ESBL and AmpC *Enterobacteriaceae* infections

- **β-lactams (BL):**
  - Carbapenems (gold standard)
  - Classic BL+BL inhibitors (BLBLI): amoxi+clavulanate, pipera-tazobactam
  - Newer BLBLI: ceftolozane+tazobactam, ceftazidime+avibactam
  - 3G-4G cephalosporins: cefotaxime/ceftriaxone, ceftazidime, cefepime
  - Temocillin
- **Non-β-lactams :**
  - Aminoglycosides
  - Fluoroquinolones
  - Cotrimoxazole
  - Tigecycline (not *Serratia/Proteus/Morganella/Providencia*)
  - Fosfomycin, nitrofurantoin (not *Serratia/Proteus/Morganella/Providencia*)

Clinical outcome in patients with ESBL-pos *Klebsiella/E.coli* bacteremia and treated with cephalosporin therapy



« In general, the exposure from usual regimens of C3 and C4 doesn't achieve the PD target for many of the ESBL organism currently classified as Susceptible »

→Clinical outcome depending on MIC level of ESBL+ isolates

D.Andes and W.Craig, Clin Microbiol Infect 2005; 11: 10-17

Outcome of ESBLE infections treated by expanded-spectrum cephalosporins

- **In vitro models:**
  - Inoculum effect*
  - Hyperexpression *bla*$_{ESBL}$ genes**
- **Clinical failure***** for low-MIC ESBL+ isolates

Table 4. Outcome in six patients treated with ceftazidime for bacteraemias caused by 'ceftazidime-susceptible' *E. coli* with CTX-M ESBLs

Sex/age (years)	Source of bacteraemia	Clinical response to ceftazidime	Etest MIC (mg/L) ceftazidime	Inhibition zones to 30 μg discs (mm)		β-Lactamase content[b]
				ceftriaxone	ceftazidime[a]	
F/70	spontaneous bacterial peritonitis	no	1	14	27/28	CTX-M-14
F/72	UTI	no	1	9	27/27	CTX-M-14, TEM-1
F/69	UTI	no	0.75 (=1)	17	31/32	CTX-M-9, TEM-1
F/83	UTI	yes	0.06	18	31/32	CTX-M-14, TEM-1
M/67	primary bacteraemia	yes	0.5	16	28/28	CTX-M-14, TEM-1
F/83	UTI	yes	0.25	15	29/28	CTX-M-14, TEM-1

➢ Caution in use of 3GC/4GC for ESBLE infections (sites, dosage)

*Wu et al. Ann Clin Microbiol Antimicob 2014
**Costa Ramos et al. JAC 2014,
***Livermore et al. JAC 2012

Clinical outcome of piperacillin-tazobactam (PTZ) vs meropenem (MEM) for C3R *E. coli* or *K. pneumoniae* BSI

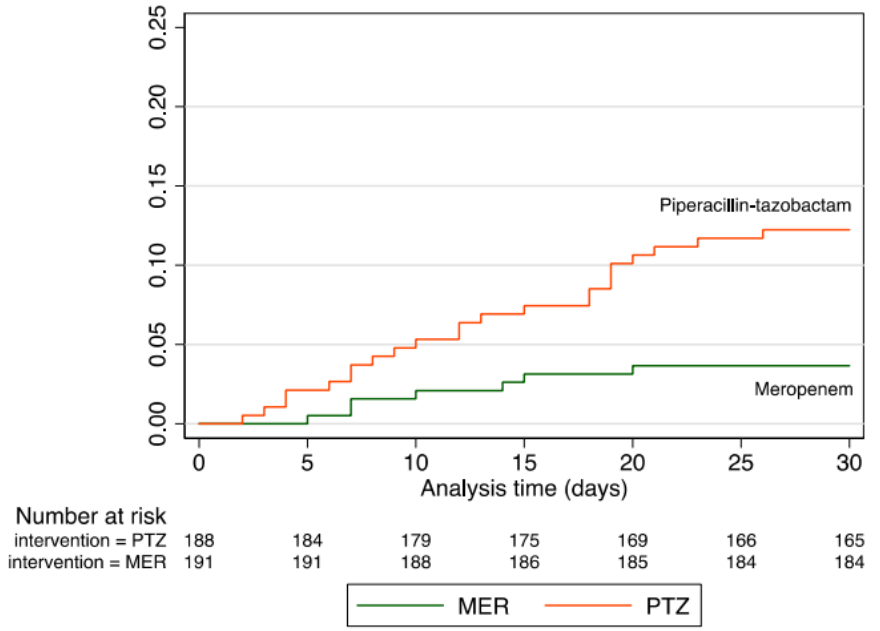
- **MERINO RCT* 30d mortality:**
  - Mortality risk difference +8.6%
    - 23 of 187 (12.3%) by PTZ
    - 7 of 191 (3.7%) by MEM
  - P = 0.90 for noninferiority.

  → Higher mortality by PTZ vs MEM

- **Pip-tazo tested by Etest diffusion!**

  → EUCAST warning**
  - Gradient tests gave variable and unreliable results
  - Warning partially lifted in April 2019

eFigure 4: Kaplan-Meier Failure Estimates for Primary Outcome



Median observation time for both meropenem (MER) and piperacillin-tazobactam (PTZ) groups = 30 days; includes primary analysis population

*Harris et al. JAMA 2018

**EUCAST warnings : http://www.eucast.org/ast_of_bacteria/warnings/

Non-susceptibility to BL/BLI combinations among ESBLE: role of OXA-1

- **OXA-1:** penicillinase resistant to BL inhibitors (weak affinity)
- **UK: 293 ESBL+ *E. coli* (ESBL-EC) from BSI:**
  - Predominant CTX-M-15 (78%), other CTX-M (21%), SHV-12 (n=3); TEM-117 (n=1)
  - **+OXA-1 for (51%;** n=149), +TEM-1 penicillinase (47%)
- **Co-carriage of OXA-1 in ESBL-EC:** resistance vs BL/BLI
  - Increased MIC to piperacillin/tazobactam (MIC50 from 2 to 8-16 mg/l) > Increased MIC to amoxi/clav (MIC50 from 4 to 8-16 mg/l)

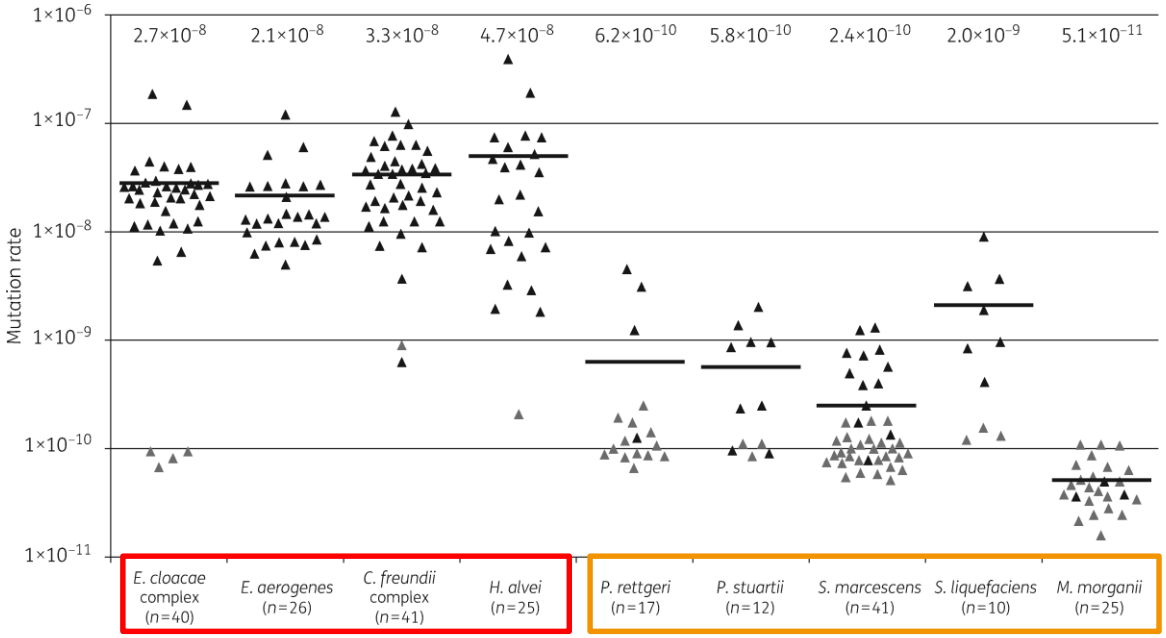
➔ OXA-1 might determine failure of BL/BLI for ESBLE treatment?

Table 2. Risk of non-susceptibility to penicillin/β-lactamase inhibitor combinations in relation to the presence of secondary β-lactamases

		Piperacillin/tazobactam				Amoxicillin/clavulanate			
	Secondary β-lactamase	relative risk of MIC >8 mg/L	95% lower CI	95% upper CI	*P*	relative risk of MIC >8 mg/L	95% lower CI	95% upper CI	*P*
All ESBL-producing *E. coli* isolates (*n* = 293)	OXA-1[a]	6.49	3.03	13.88	<0.001	2.34	1.85	2.96	<0.001
	TEM-1/191	1.32	0.81	2.14	0.257	1.00	0.82	1.22	0.992
	OXA-1 + TEM-1/191	3.49	2.22	5.48	<0.001	1.72	1.47	2.02	<0.001

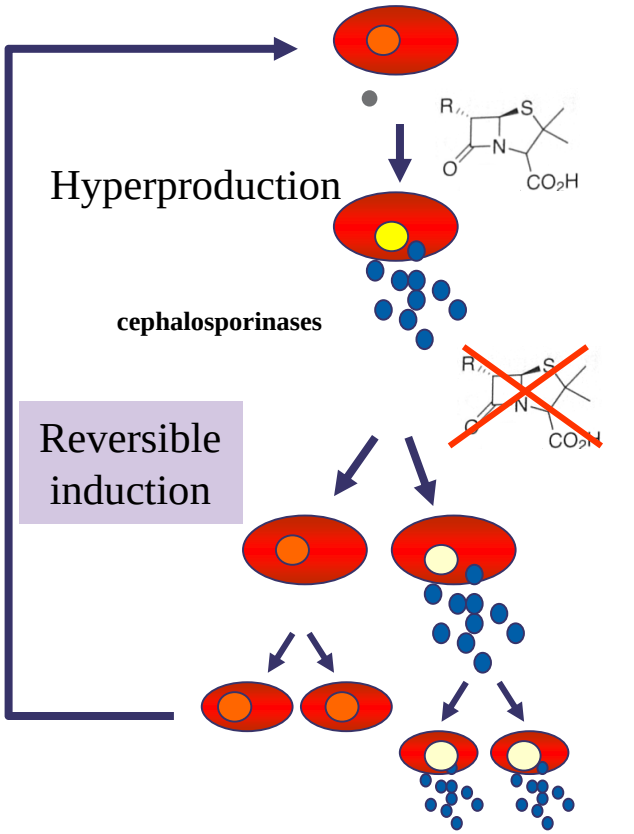
Risk of derepressed AmpC (hyperproduced cephalosporinase)

- Hyperproduction of chromosomal derepressed AmpC
  - → 3GC-R and Pip-tazo-R
  - Usually S to 4GC, carbapenems, temocillin
- **In vitro species-specific mutation rates:**



High mutation rates

Lower mutation rates



Risk of derepressed AmpC (hyperproduced cephalosporinase)

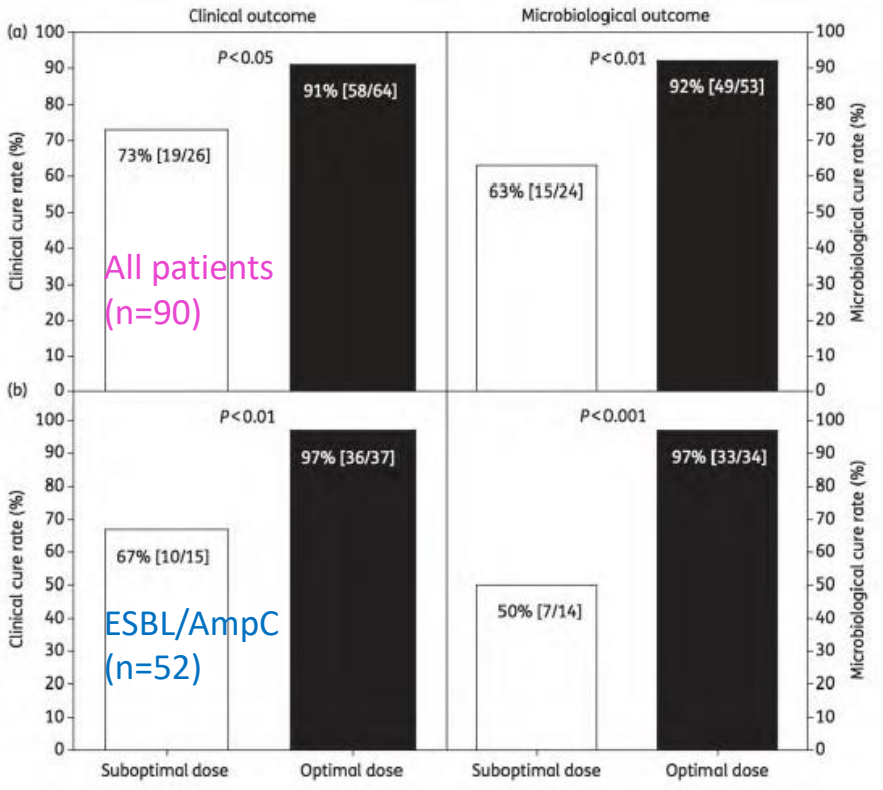
- **Clinical emergence of R with 3GC** * on WT *Enterobacterales* with inducible AmpC:
  - *Enterobacter* spp: 8%-19%
  - *S. marcescens* and *M. morganii*: 0-3%
- **EUCAST expert rules** v3.2 (June 2019)**: IF in vitro S to 3GC (cefotaxime, ceftriaxone or ceftazidime), THEN note that
  - **For *Enterobacter spp, Citrobacter freundii cplx, Hafnia alvei*:**
    - monotherapy with 3GC as well as combination therapy of these agents with an aminoglycoside should be discouraged owing to risk of selecting resistance,
    - OR suppress the susceptibility testing results for these agents
  - **For *Serratia spp, Morganella morganii, Providencia spp*:**
    - that monotherapy with 3GC may infrequently select resistant mutants

*Choi et al. AAC 2008; Kohlmann et al. JAC 2018

**http://www.eucast.org : Enterobacterales_ExpertRules_V3.2_20190613.pdf

Temocillin

- 6-α-methoxy-ticarcillin
- Belgium, UK (1980s'); France, Germany
- Activity spectrum:
  - mainly limited to *Enterobacteriales*
  - stable to β-lactamases including ESBL, AmpC
- Dosage: UD 2x 2g IV, HD 3x 2g IV/CI
- Clinical data:
  - UK[1]: 92 patients (41 UTI and 42 BSI)
  - Belgium[2]: 3rd AB used in UTI in hospitals
- Microbiological data:
  - MIC50/MIC90 remained at 8/16 mg/L over the last 20 years
  - EARS Belgium 2018 (Blood/Urine) [3]:
    - Temo-R <4% in *E. coli*; <6% in *K. pneumoniae*
- Upcoming EUCAST breakpoints…
  - Proposal S≤0.001, R>16 mg/l with only HD 3x 2g!?!?



Breakpoints (mg/l)	infection	S≤	R>
Fuchs EJCM 1985		16	16
BSAC, Vanstone JAC 2013	systemic	8	8
	uUTI	32	32

[1]Balakrishnan et al. JAC 2011; [2]Versporten et al. ECCMID 2019 P2045; [3]EARS-Net data 2018

CARBAPENEM RESISTANCE: MECHANISMS

Enterobacteriaceae	Cephalosporinase/ESBL + porin loss Carbapenemase
P. aeruginosa	Porin loss Up-regulated efflux Carbapenemase
Acinetobacter spp.	Cephalosporinase + porin loss Carbapenemase

Ambler classification of carbapenemases

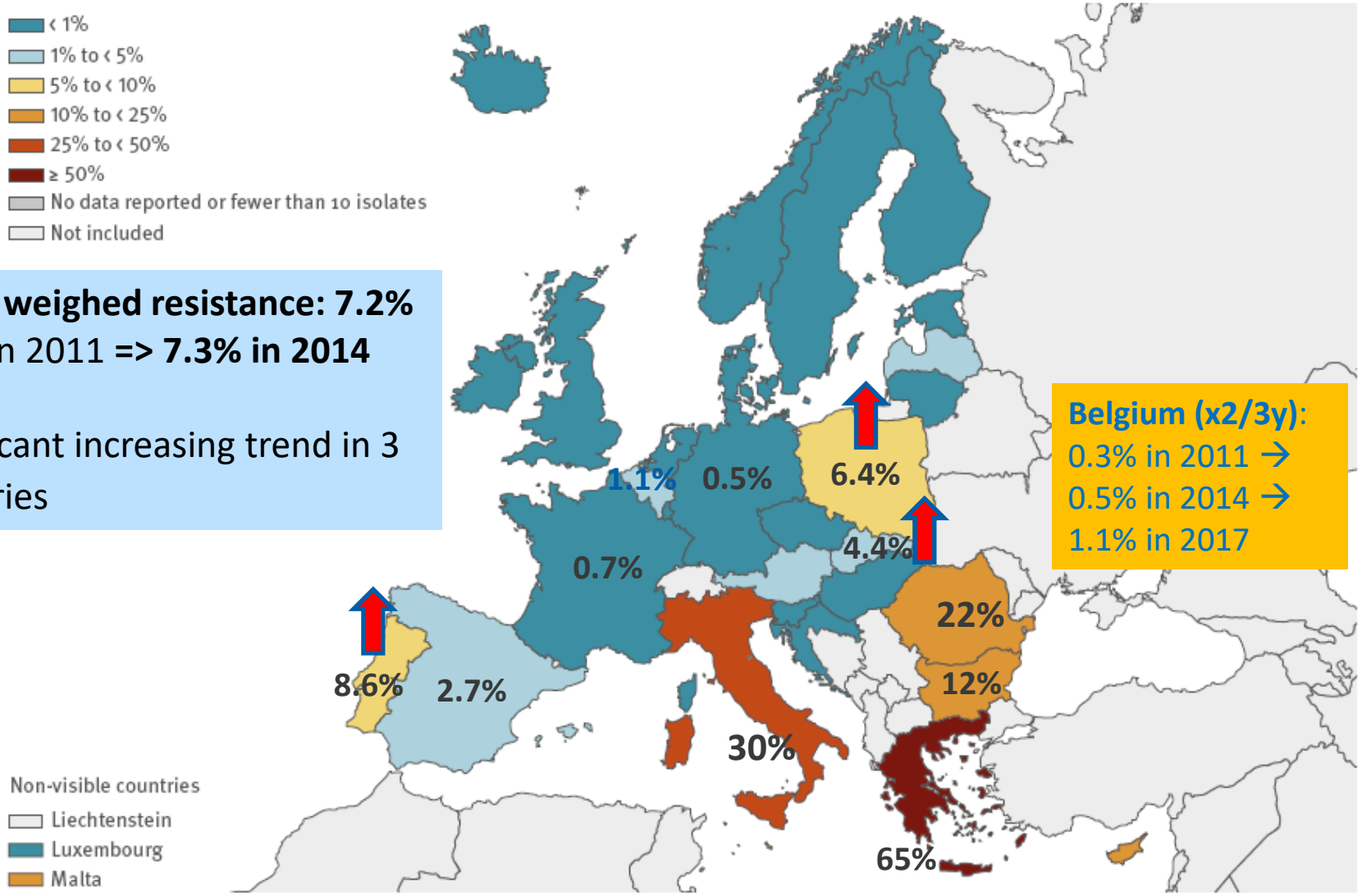
Ambler molecular class	In vitro inhibitors	Carbapenemase types
A	PBA	**KPC**
		GES
		IMI, SME, NMC…
B (MBL)	DPA	**VIM, IMP, NDM**
		GIM, SIM, SPM…
D	-	**OXA-48-like**
		OXA-23-, OXA-24-, OXA-58-like
		OXA-198, OXA-372, OXA-427…

MBL (metallo-β-lactamase); PBA (phenylboronic acid); DPA (dipicolinic acid)

- Resistance to most β-lactams
- Some associations organisms-carbapenemase types
- **Class-specific (A, B) inhibitors of carbapenemases**

→ **Major carbapenemases in *Enterobacteriaceae* (CPE)**

Proportion of invasive *K. pneumoniae* isolates resistant to carbapenems in Europe (2017)

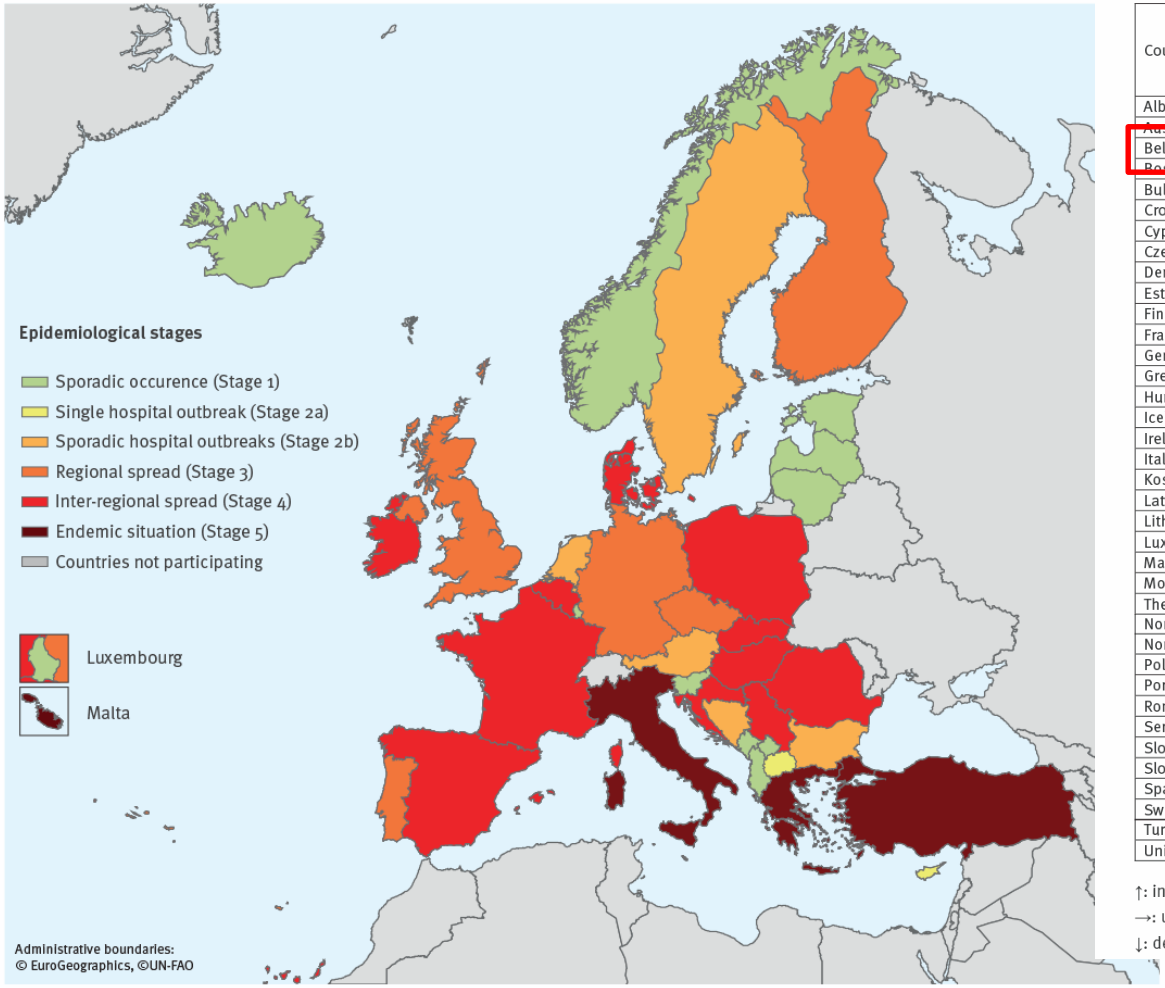


Mean weighed resistance: 7.2%
6.0% in 2011 **=> 7.3% in 2014**

Significant increasing trend in 3 countries

Belgium (x2/3y):
0.3% in 2011 →
0.5% in 2014 →
1.1% in 2017

Epidemiological stage of CPE in Europe, July 2018 (n = 37)

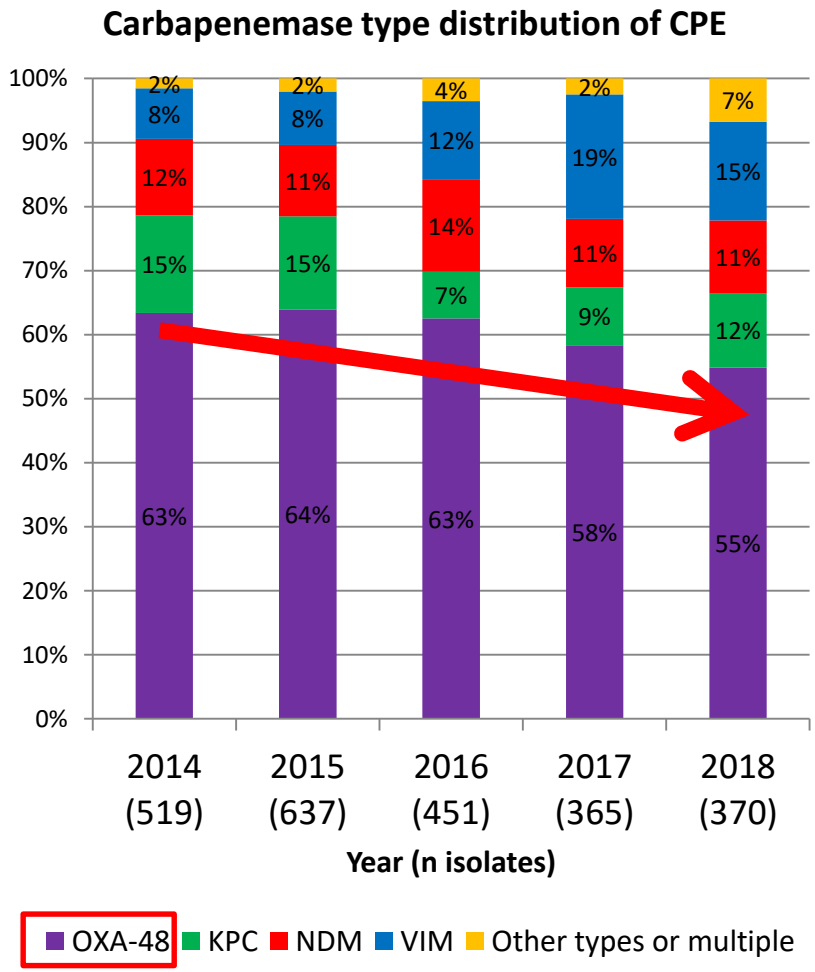


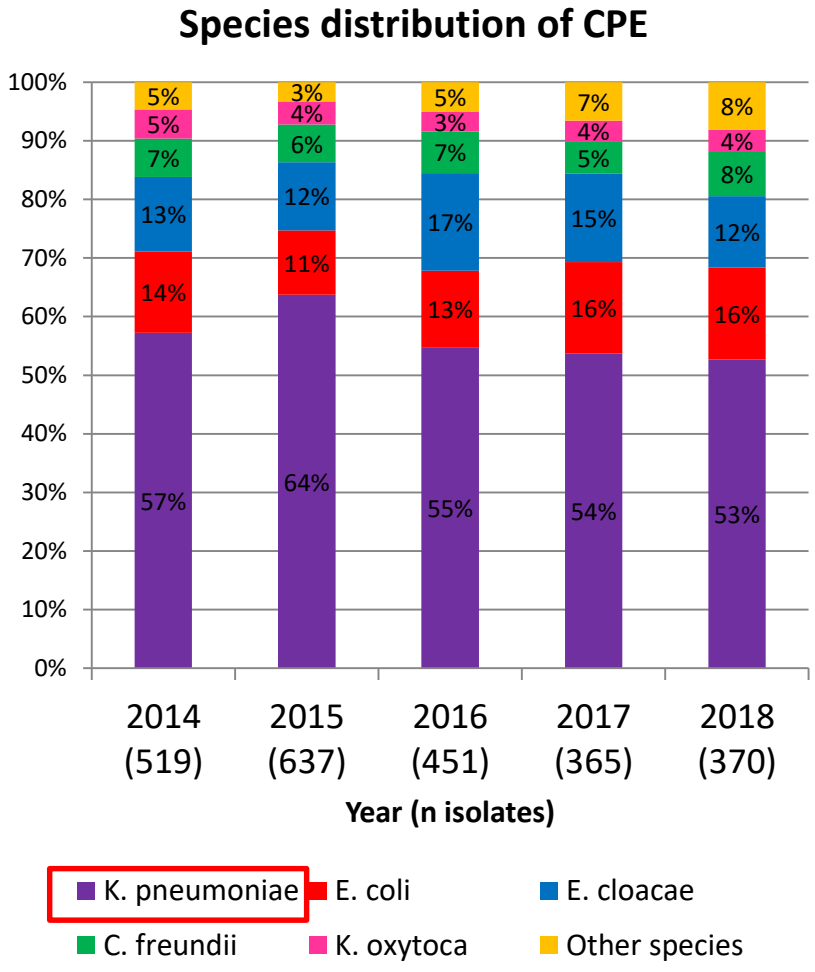
Country	Epidemiological stage for the spread of carbapenemase-producing Enterobacteriaceae				Change in epidemiological stage 2015–18
	2010 [11]	2013 [9]	2014–15 [8]	2018	
Albania	NA	2a	1	1	→
Austria	0	2b	2b	2b	→
Belgium	2b	3	4	4	→
Bosnia and Herzegovina[a]	1	1	0	2b	↑
Bulgaria	0	2a	2a	2b	→
Croatia	1	3	3	4	↑
Cyprus	2a	2a	1	2a	↑
Czech Republic	1	2b	2b	3	↑
Denmark	1	2a	4	4	→
Estonia	0	2a	1	1	→
Finland	1	2a	2a	3	↑
France	3	3	4	4	→
Germany	3	3	3	3	→
Greece	5	5	5	5	→
Hungary	3	4	4	4	→
Iceland	0	0	0	1	↑
Ireland	1	4	3	4	↑
Italy	4	5	5	5	→
Kosovo[b]	NA	2b	0	1	↑
Latvia	1	1	1	1	→
Lithuania	1	1	1	1	→
Luxembourg	NA	1	1	1	→
Malta	1	5	5	5	→
Montenegro	NA	0	1	1	→
The Netherlands	2a	2b	2a	2b	→
North Macedonia	NA	0	1	2a	↑
Norway	2a	2a	1	1	→
Poland	4	3	4	4	→
Portugal	1	1	2b	3	↑
Romania	1	1	4	4	→
Serbia	1	1	2b	4	↑
Slovak Republic	NA	2a	4	4	→
Slovenia	0	1	2a	1	↓
Spain	2b	3	4	4	→
Sweden	2a	2b	2a	2b	→
Turkey	NA	2a	5	5	→
United Kingdom[c]	2b	3	3	3	→

↑: increase in the epidemiological stage between 2015 and 2018
→: unchanged epidemiological stage between 2015 and 2018
↓: decreased epidemiological stage between 2015 and 2018

Brolund et al. Euro Surveill. 2019;24(9):pii=1900123.

Carbapenemase types and species distribution among CPE



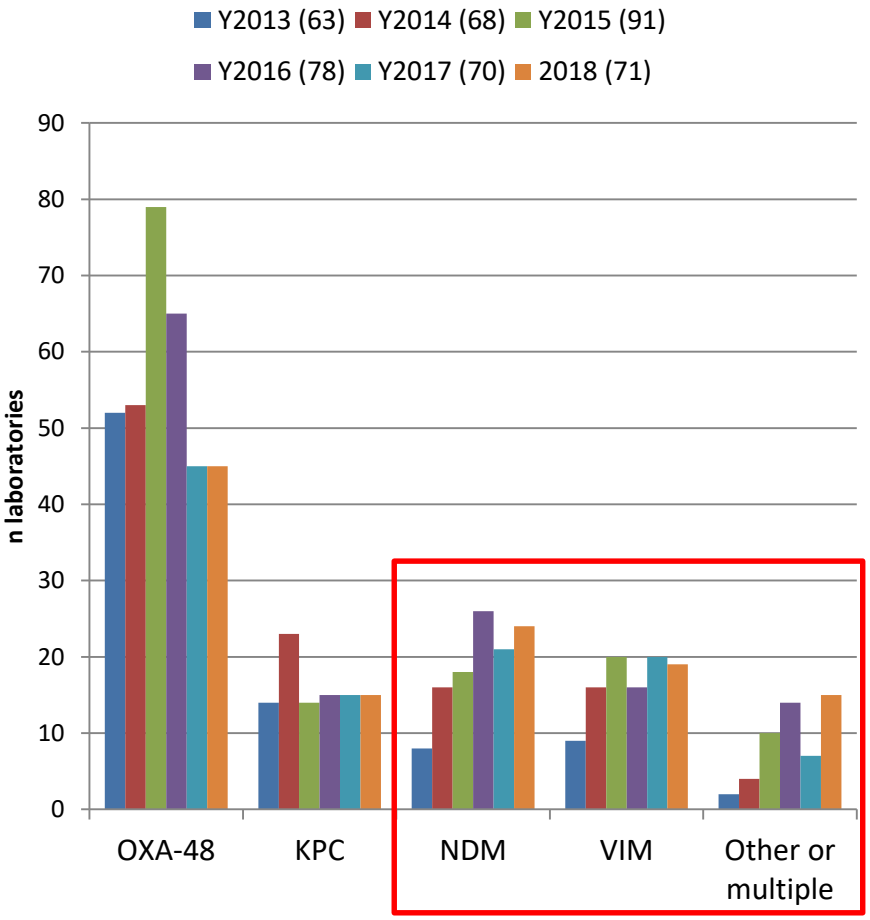


➢ ***K. pneumoniae*** and **OXA-48**: predominant CPE species and carbapenemase types

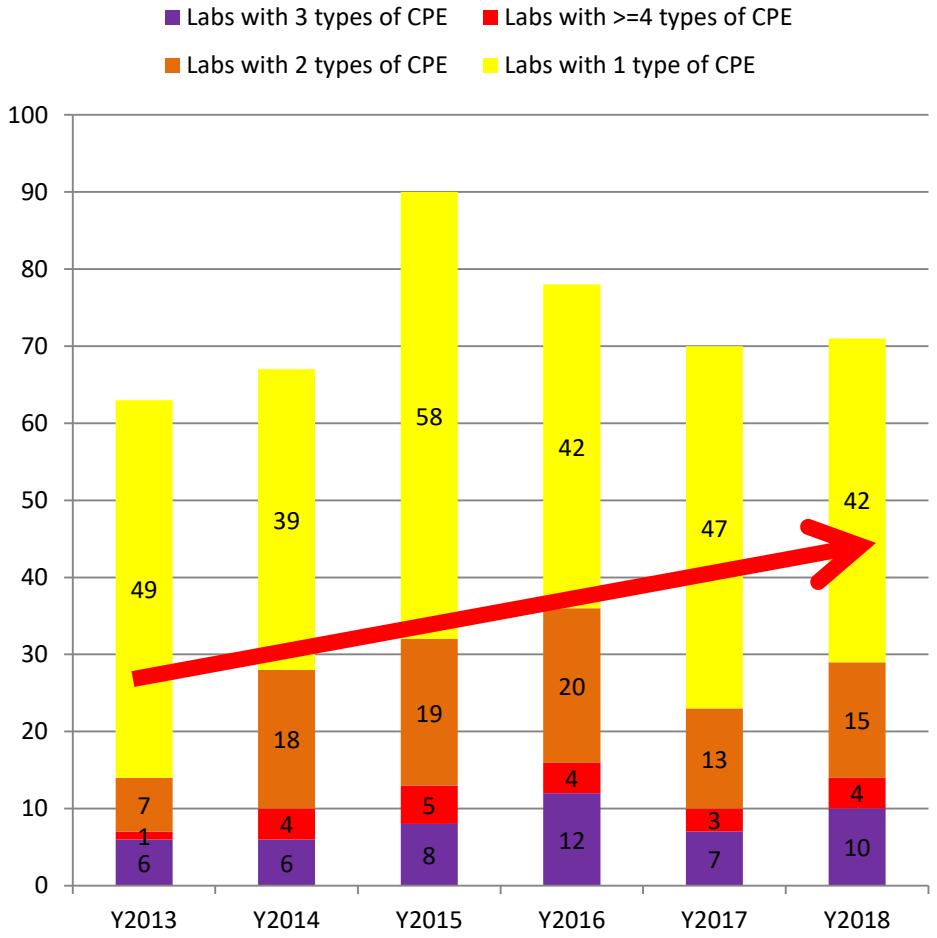
→ **Diversification of enzyme and of species** among confirmed CPE

Diversity of carbapenemases among CPE in Belgium

Carbapenemases types in CPE (laboratories)

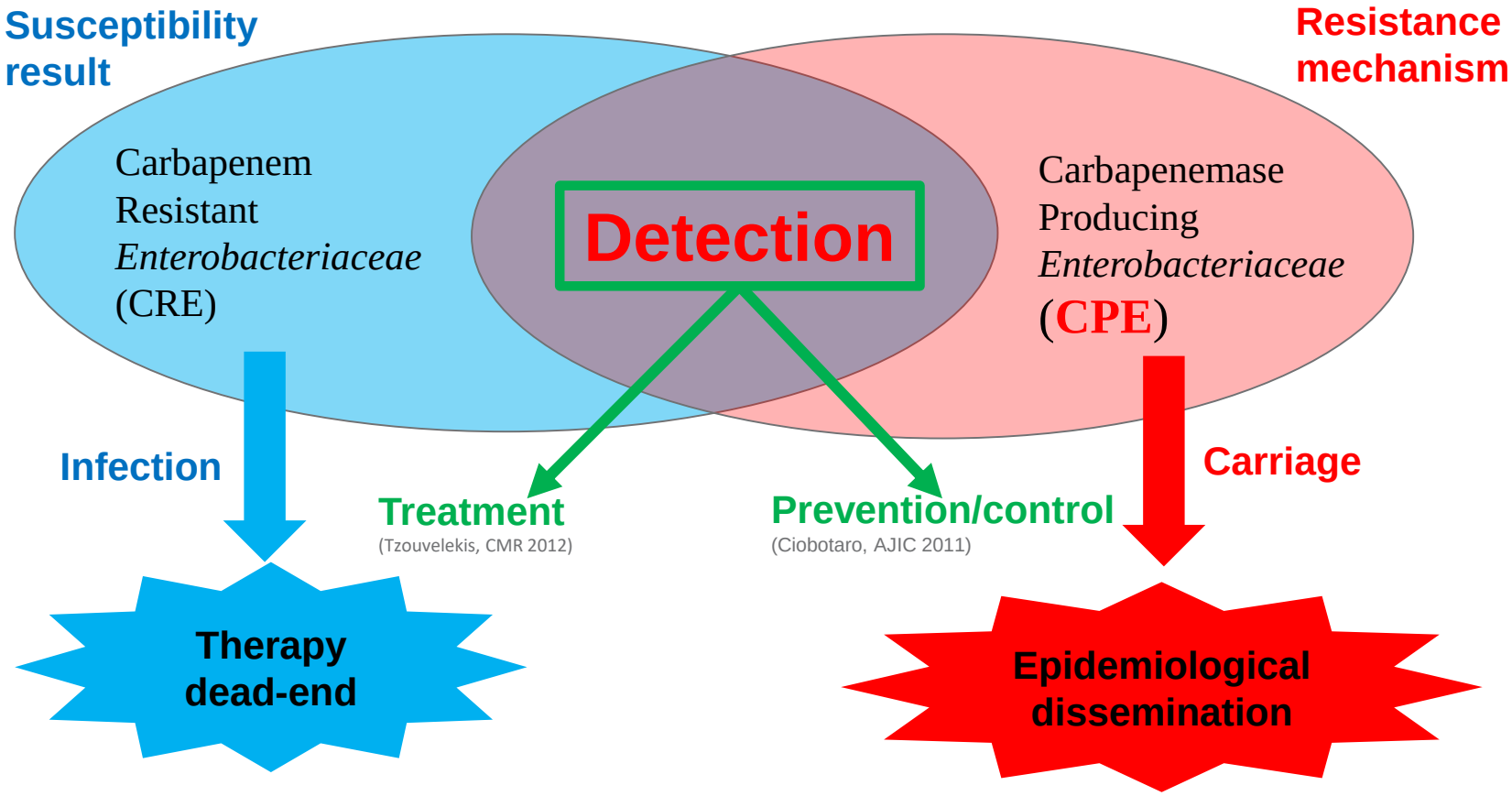


Number of carbapenemase types in CPE (laboratories)



- Increase and spread of **NDM** CPE
- **Diversification** with spread of multiple carbapenemase types of CPE

Clinical implication of CPE detection



- Fast and accurate diagnostic is central in the management of carbapenem resistance

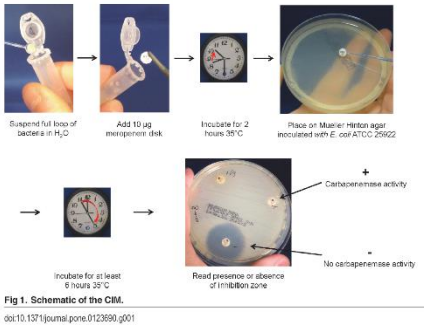
Purposes for CPE detection tests

Test purpose	Turnaround time	Information needed	Capacity for testing
Therapeutic decision	**Rapid** (1 day)	**Susceptibility results to guide antimicrobial selection**	**All clinical microbiology laboratories**
		Detect all clinically relevant antimicrobial resistance	
		Identify major carbapenemase types to guide antimicrobial selection	
		Avoid overreporting resistance	
Infection control decisions	**Rapid** (1–2 days)	**Detect epidemiologically important antimicrobial resistance including major carbapenemases**	
		Detect all carbapenemases	Reference laboratories
Epidemiology description	Slow (can be batched)	Confirmation of facility-level results	Reference laboratories
		Identify specific emerging resistance mechanisms	State or public health laboratories

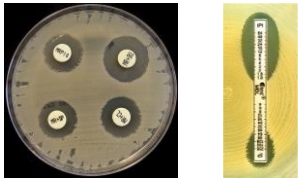
Detection methods of CPE on cultured colonies

Phenotypic: 24h

CIM test



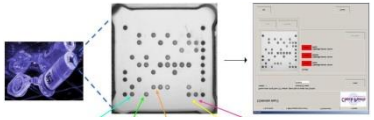
Inhibition tests



Molecular

PCR et RT-PCR < 1h

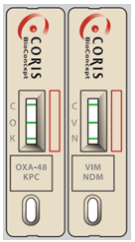
DNA micro-array 7h

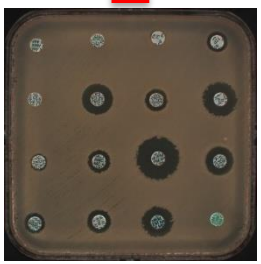


NGS >2d

Immuno-chromatography: 15’

OXA-48
KPC
NDM
VIM
IMP

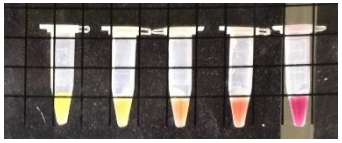


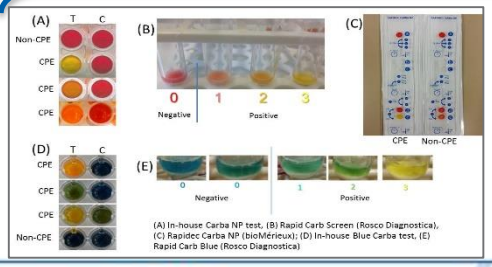


Carbapenem hydrolysis

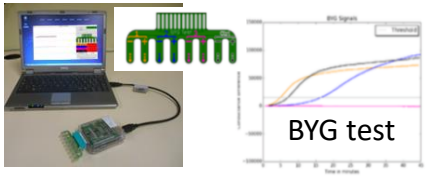
Colorimetry 30m-2h

↓pH or chromogenic

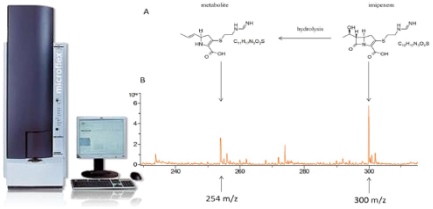




Electro-chemistry 30’



MALDI-TOF 30’



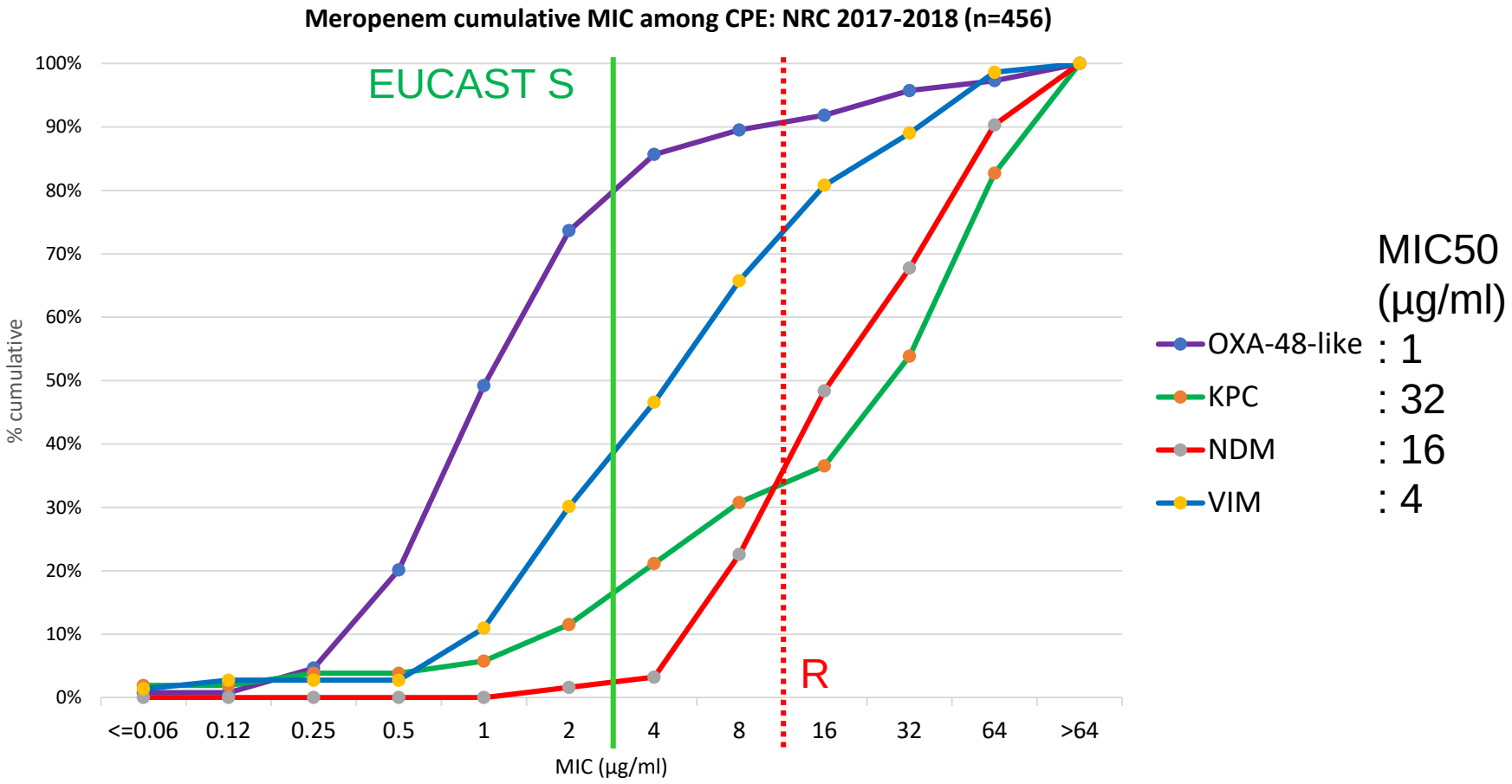
Treatment of CRE / CPE infections

- **β-lactams (BL):** available and awaiting new drugs or combinations
  - Meropenem: extended infusion (if MIC ≤8 mg/l)
  - 3G-4G cephalo (ceftazidime, cefepime): OXA-48 if not ESBL or AmpC producers
  - Aztreonam: MBL (VIM, NDM…) if not ESBL or AmpC producers
  - BL+BL inhibitors (ceftazidime+avibactam): most KPC and OXA-48
- **Non-β-lactams :**
  - Aminoglycosides
  - Fluoroquinolones
  - Cotrimoxazole
  - Colistin (not *Serratia/Proteus/Morganella/Providencia*)
  - Tigecycline (not *Serratia/Proteus/Morganella/Providencia*)
  - Fosfomycin, nitrofurantoin (not *Serratia/Proteus/Morganella/Providencia*)

Susceptibility data for CPE
Belgian NRC 2017-2018 (n=476)

EUCAST %S by BMD	OXA-48-like	KPC	NDM	VIM
Total n	259	52	62	73
Temocillin (Vanstone JAC 2013)	1%	2%	3%	0%
Piperacillin/tazobactam	1%	0%	0%	0%
Aztreonam	41%	0%	18%	42%
Cefotaxime	26%	0%	0%	0%
Ceftazidime	40%	0%	0%	0%
Cefepime	40%	0%	0%	3%
Meropenem	74%	12%	2%	30%
Ceftolozane/tazobactam	22%	0%	0%	0%
Ceftazidime/avibactam	100%	100%	0%	6%
Ciprofloxacin	26%	6%	3%	18%
Gentamicin	65%	46%	27%	51%
Amikacin	95%	27%	32%	85%
Fosfomycin	71%	46%	82%	85%
Tigecycline	90%	90%	84%	96%
Colistin	92%	78%	87%	94%

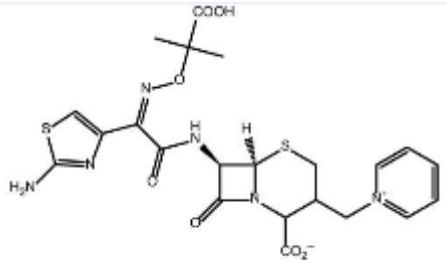
Meropenem cumulative MIC distribution among CPE



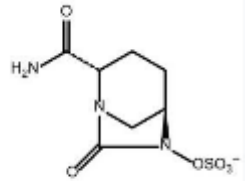
➢ **Lower MIC50** (mg/L) **in OXA-48** (1) and **VIM** (4) than in KPC (32) and NDM (16) CPE

Existing ß-lactams +avibactam (Old/new)

- **Ceftazidime +avibactam** (Zavicefta®, Avycaz®)
  2g/0.5g /8h ; costs 318€/day
- Activity spectrum*:
  - Active on ESBL, AmpC and some CPE (KPC, OXA-48)
  - **Inactive on MBLs (VIM, IMP, NDM)**
  - No activity on Gram+ and anaerobes
- EUCAST/CLSI breakpoint S ≤8 µg/ml
- In vitro activity CAZ-AVB**:
  - >97% vs consecutive *Enterobacteriaceae* including ESBLE and CRE (mainly KPC) and *P. aeruginosa*
  - <62% vs MDR *P. aeruginosa*



ceftazidime



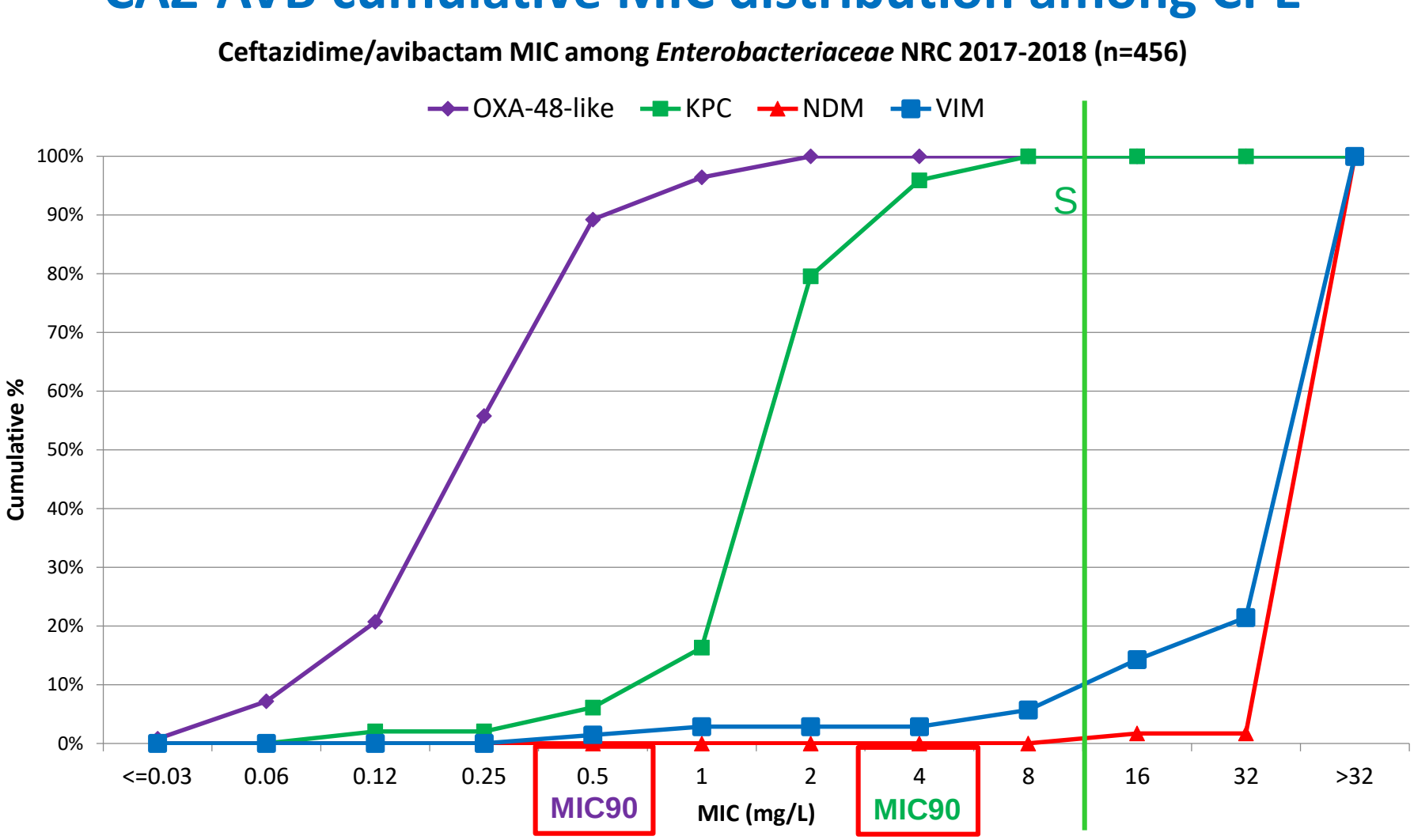
avibactam

FDA approved 25 Feb 2015; EMA approved 28 April 2016

- Comblicated intra-abdominal Infections;
- Complicated urinary tract infections (including pyelonephritis);
- Hospital acquired pneumonia (including VAP)
- Infection by resistant Gram-neg with limited therapeutic options

*Lucasti et al. JAC 2013; Riccobene et al. AAC 2013
**Sader et al. AAC 2017; Humphries et al. AAC 2017

CAZ-AVB cumulative MIC distribution among CPE



- Higher CAZ-AVB MIC for **KPC** than for OXA-48 CPE

AST methods for CAZ-AVB

- **Clinical breakpoints:**

Methods	*Enterobacteriales* (S≤; R>)	*Pseudomonas* (S≥; R<)
MIC	8 mg/l	8 mg/l
EUCAST disk 10/4 µg	13 mm	17 mm
CLSI disk 30/20 µg	21 mm	17 mm

- **Performance of Etest and disk diffusion vs BMD (broth microdilution):**
  - N=103 CRGNB*: No VME, high CA for Etest and 10/4µg disk only for *K. pneumoniae*

Table 1. Performance of Etest and discs compared with BMD for evaluated Gram-negative isolates

Method	EA (%)[a]	CA (%)	MEs (%)	VMEs (%)
Meropenem and ceftazidime non-susceptible *K. pneumoniae* isolates ($n = 69$)				
Etest	82.4[e]	100	0	0
30/20 µg disc	–	82.6	18.5	0
10/4 µg disc	–	98.5	1.5	0
MDR non-*K. pneumoniae* isolates ($n = 33$)[f]				
Etest	64.3[g]	84.8	33.3	0
30/20 µg disc	–	75.8	53.3	0
10/4 µg disc	–	63.6	80	0

  - ➢ Prefer MIC by BMD and Etest as alternative for CAZ-AVB testing

*Wenzler et al. JAC 2019

Resistance mechanisms to ceftazidime-avibactam

- **Carbapenemase mutations:**
  - KPC-3 vs KPC-2 (20x higher catalytic hydrolysis of ceftazidime → MIC x10)
  - Increased expression of KPC-3 (transposition of KPC Tn4401 in a second plasmid →higher genes copy number)
  - **KPC mutants** observed in vitro and during CAZ-AVB treatment
    - Mutations in the KPC Ω loop active site: D179Y
    - CARBA → ESBL phenotype: ceftazidime hydrolysis x100-1000 → CAZ-AVB MIC x16… and carba-S
- **ESBL and AmpC mutations:**
  - *K. pneumoniae* OXA-48 and mutated CTX-M-14 → MIC x16
  - In vitro PER-1 ESBL *P. aeruginosa*, in vitro mutants CTX-M-15 ESBL
  - AmpC hypermutants *P. aeruginosa* CAZ-AVB-R (G183D)
- **Porin mutations** → decreased permeability to CAZ-AVB:
  - Previous treatment with cefepime/carbapenem → mutations in OmpK36 porin (Klebsiella spp., Enterobacter spp.)

Livermore et al. AAC 2015, Compaire et al. AAC 2017, Haisdar et al. AAC 2017, Humphries et al. AAC 2017, Both et aL. JAC 2017, Gaibani et al. JAC 2018, Mueller et al. 2019, Poirel et al. JAC 2019, Livermore et al. JAC 2019, Boulant et al. AAC 2019, Khil et al. mBio 2019

In vivo development of CAZ-AVB resistance

KP KPC-3 D179Y (ST1519)

MIC (mg/l)
CST >16
MEM ≥32
CAZAVB =8

MIC (mg/l)
CST >16
MEM =8
CAZAVB ≥256

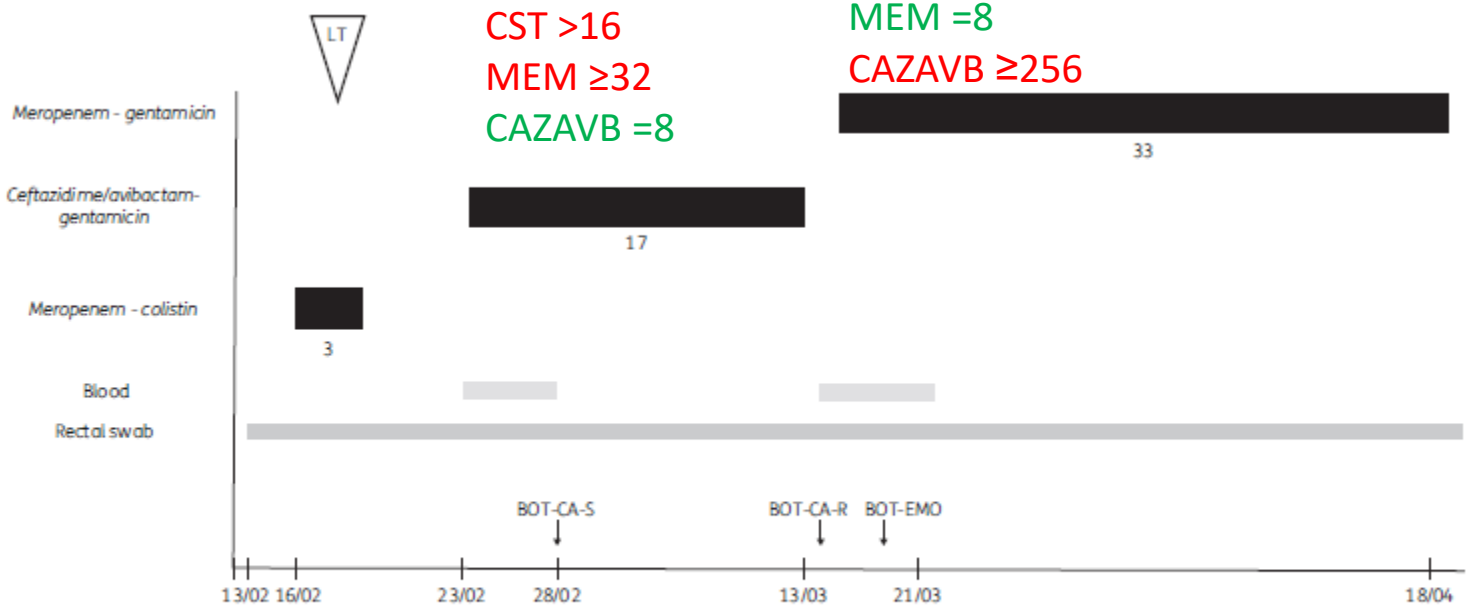


Figure 1. History of the isolation and antimicrobial treatments of a patient with KPC-Kp infections. Black bars represent the antimicrobial treatments and numbers indicate the days of therapy. Grey bars represent the duration of bacteraemia episodes and persistent colonization by KPC-Kp. The triangle indicates the time of liver transplant (LT) and arrows show the isolation times of BOT-CA-S, BOT-CA-R and BOT-EMO.

CAZ-AVB resistance by change of epidemiology

- **Epidemiology reversal** of carbapenemase-producing *K. pneumoniae* from KPC- to VIM producers in a Greek ICU after introduction of ceftazidime/avibactam

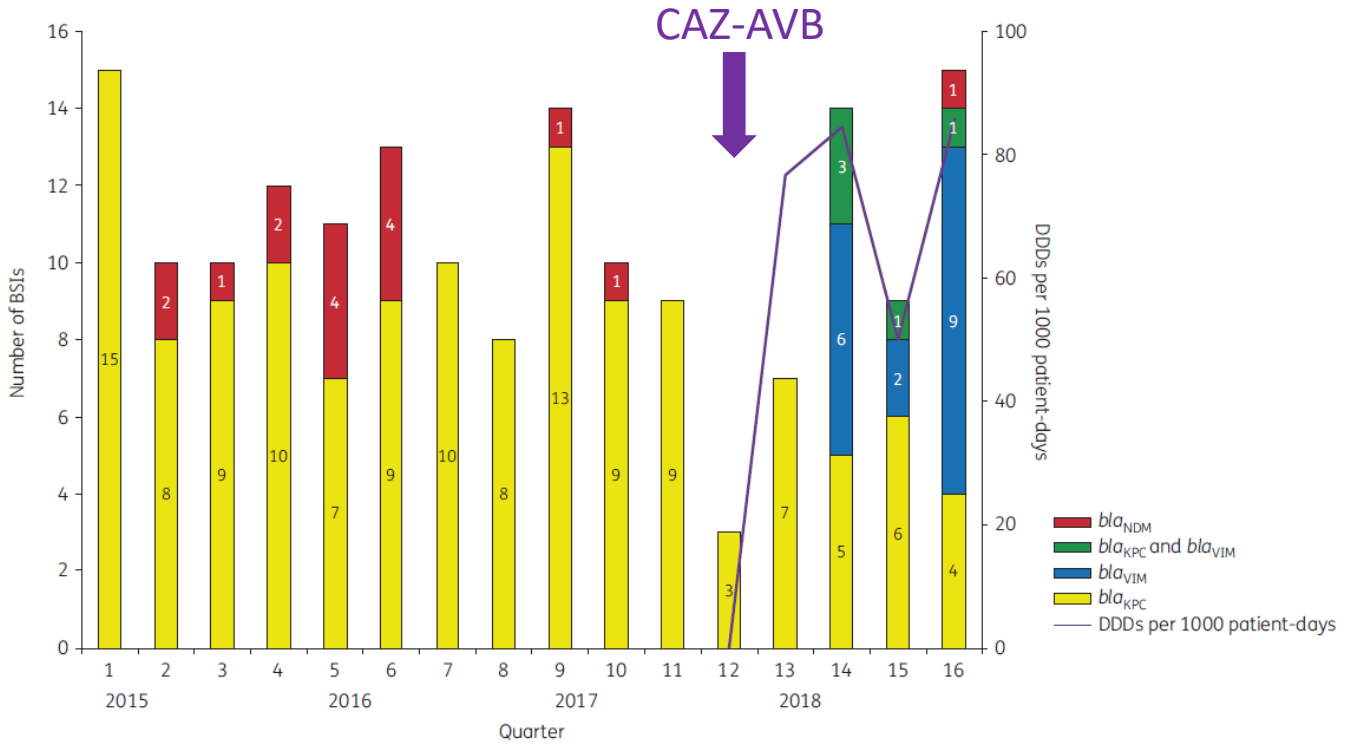


Figure 1. Quarterly distribution of carbapenemase genes among CP-Kp BSIs and ceftazidime/avibactam consumption (DDDs per 1000 patient-days). This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Papadimitriou-Olivgeris et al. JAC 2019

Aztreonam / Avibactam

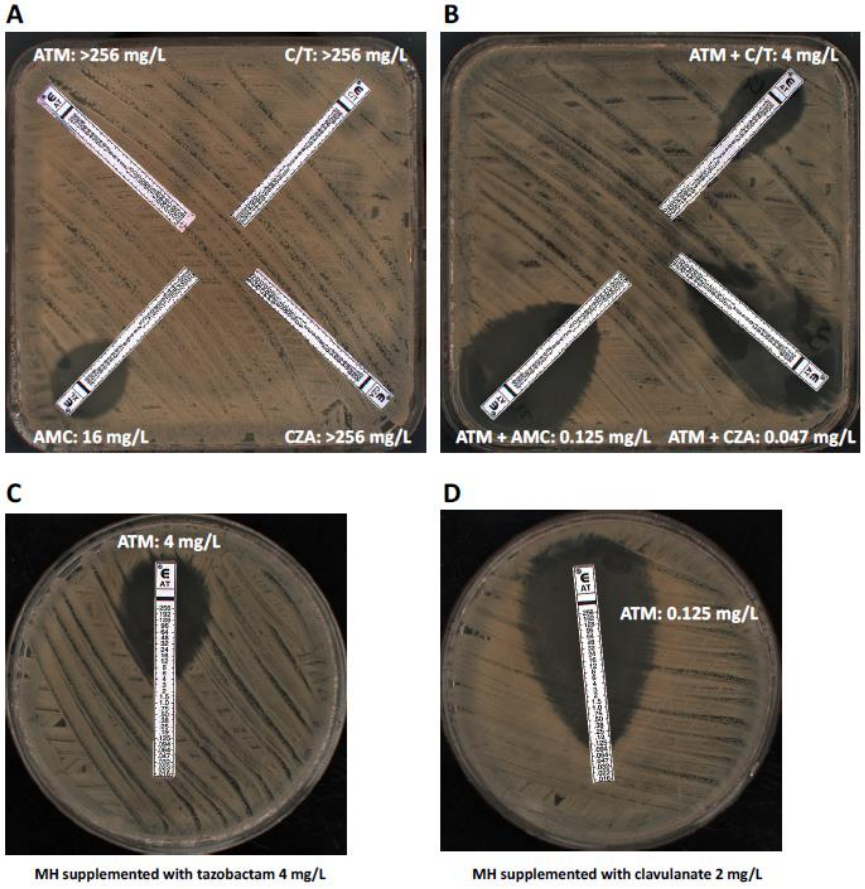
- **Rationale**:
  - **Aztreonam** (AZT) hydrolyzed by many serine β-lactamases (Class A ESBLs (CTX-Ms, TEM, SHV) and CPE KPC), but remains active against MBLs (Class B)
  - Combination **AZT+avibactam** (AZT-AVB) → activity against MDR including carbapenemases (OXA-48, KPC and **MBLs) associated to ESBL/AmpC**
- **Activity of AZT-AVB:**
  - MIC_{90} of AZT-AVIB (4 mg/L)= 0.12 µg/ml for all Gram-negative (large US survey*)
  - Overall 99,9% susceptibility vs *Enterobacteriaceae* (most CRE with MIC < 2 µg/ml)
  - Less active vs MDR *P. aeruginosa* isolates (MIC90: 32-64 µg/ml);
  - No activity vs *A. baumannii*
- **Clinical data:**
  - Phase II clinical trial in cIAI ongoing
  - Successful treatment outcomes by combining CAZ/AVB + AZT for MDR Gram-neg**:
    - MBL carbapenemase producers
    - Compassionate usage in small number of patients, no randomized controlled trial

*Karlowsky et al. AAC 2017
**Davido et al. AAC 2017

Association of aztreonam + clavulanate or ceftazidime/avibactam for MBL producer

- **Successful treatment of 2 clinical cases:**
  - UTI by NDM CPE + CTX-M ESBL *E. coli* isolate R/ aztreonam-ceftazidime-avibactam (AZT+CAZ-AVB)
  - Pneumonia by XDR *S. maltophilia* R/ aztreonam-amoxicillin-clavulanate (AZT+AMX-CLA)
- **In vitro evaluation on MBL producers:**
  - AZT testing with CAZ-AVB or AMX-CLA by Etest strip superposition method
  - 50 **EB**: 86% S to AZT+CAZ-AVB and 50% to AZT+AMX-CLA
  - 3 **PA**: AZT+CAZ-AVB but MICs reduction max -2x
  - 5 ***S. maltophilia***: 100% S to AZT+CAZ-AVB and AZT+AMX-CLA
- **Conclusions:**
  - AZT + CAZ-AVB the most potent combination
  - AZT + AMX-CLA as cheaper alternative
  - Etest superposition to test AZT combinations

NDM-1 E. coli



Emeraud et al. AAC 2018

Back to the basics…