

# Carbapenem resistance in *Enterobacterales*: impact of new screening cut-off

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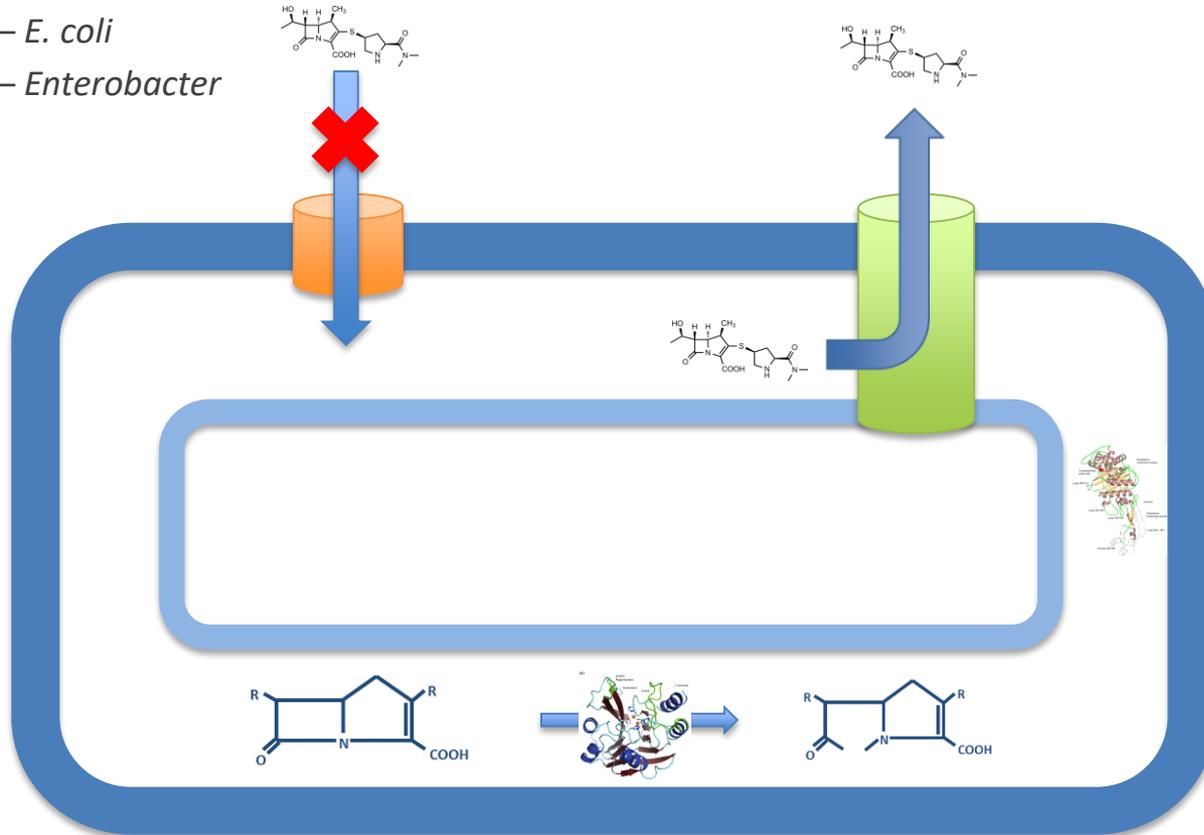
# MECHANISMS OF CARBAPENEM RESISTANCE - *Enterobacterales*

## ➤ Reduced permeability

- OmpK36 – *K. pneumoniae*
- OmpC/F – *E. coli*
- OmpC/F – *Enterobacter*

## ➤ Active efflux

- AcrAB-TolC - *Enterobacteriaceae*



## ➤ PBP mutations

- PBP-2 (*E. coli*)

## ➤ β-lactamases:

- **CARBAPENEMASES**
- ESBL/AmpC + reduced permeability

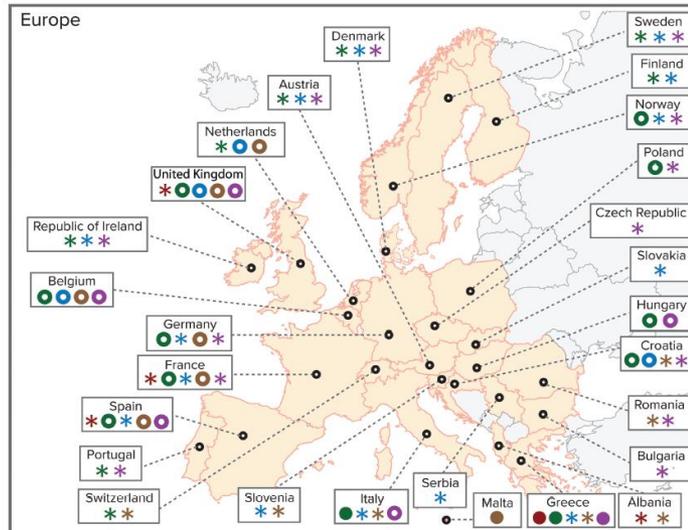
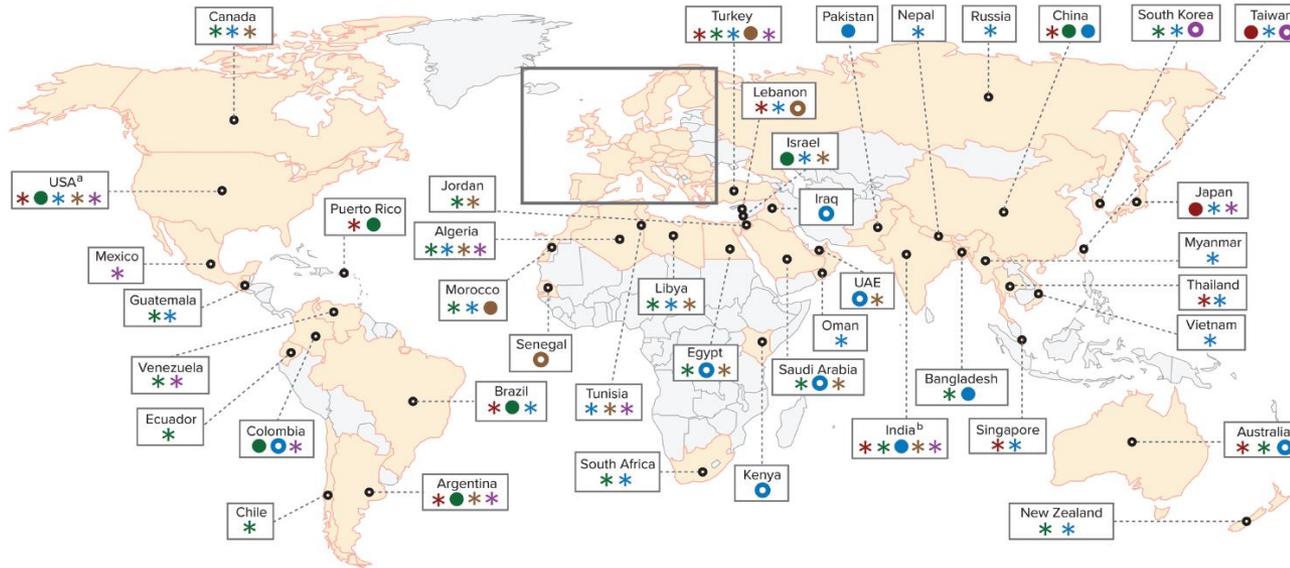
# WHY SCREENING?

- Detection of carbapenemase-producing *Enterobacterales* (CPE)
- Carbapenemases:
  - Confer resistance/reduced susceptibility to  $\beta$ -lactams including carbapenems
  - Carbapenemase genes – plasmid-mediated -> readily transferable
  - CPE isolates frequently resistant to non- $\beta$ -lactam antibiotics ->MDR
  - Infections with CPE associated with high mortality rates

Importance of detection of resistance mechanism	
Required for <b>clinical</b> antimicrobial susceptibility categorization	No
Infection control <b>purposes</b>	Yes
Public health <b>purposes</b>	Yes



# CPE – GLOBALLY DISTRIBUTED

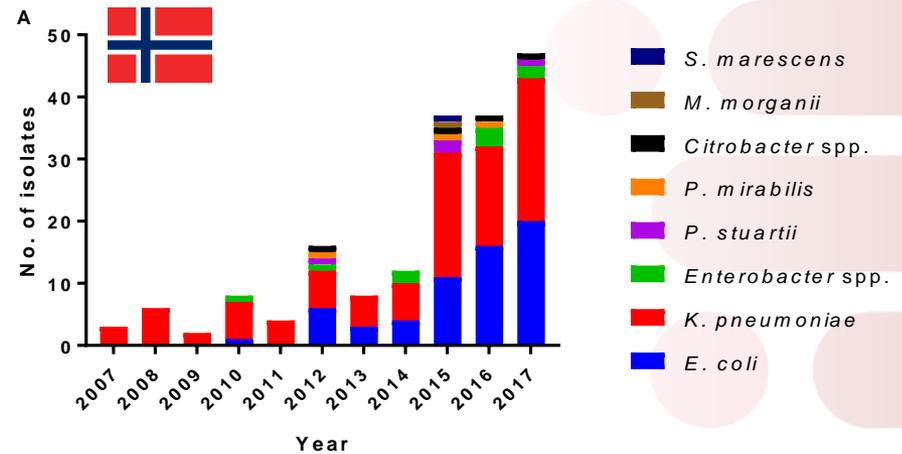
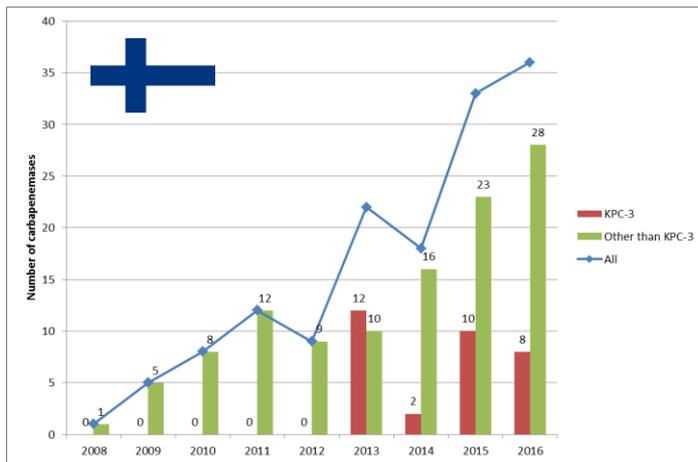
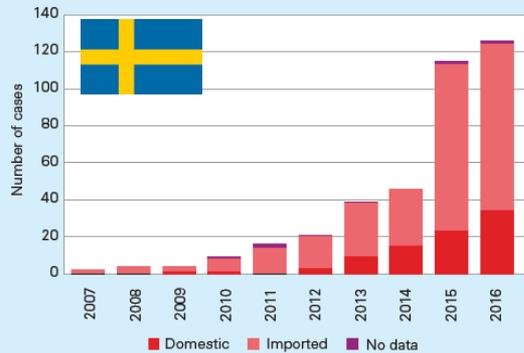


	IMP	KPC	NDM	OXA	VIM
Endemic/nationwide distribution	●	●	●	●	●
Significant outbreaks/regional spread	○	○	○	○	○
Sporadic outbreak/occurrences	*	*	*	*	*



# CPE NORDIC COUNTRIES

**FIGURE 3.5.** Number of human cases of ESBL<sup>CA</sup> annually notified as domestic and imported in Sweden, 2007-2016.



Sporadic cases



# CARBAPENEMASES

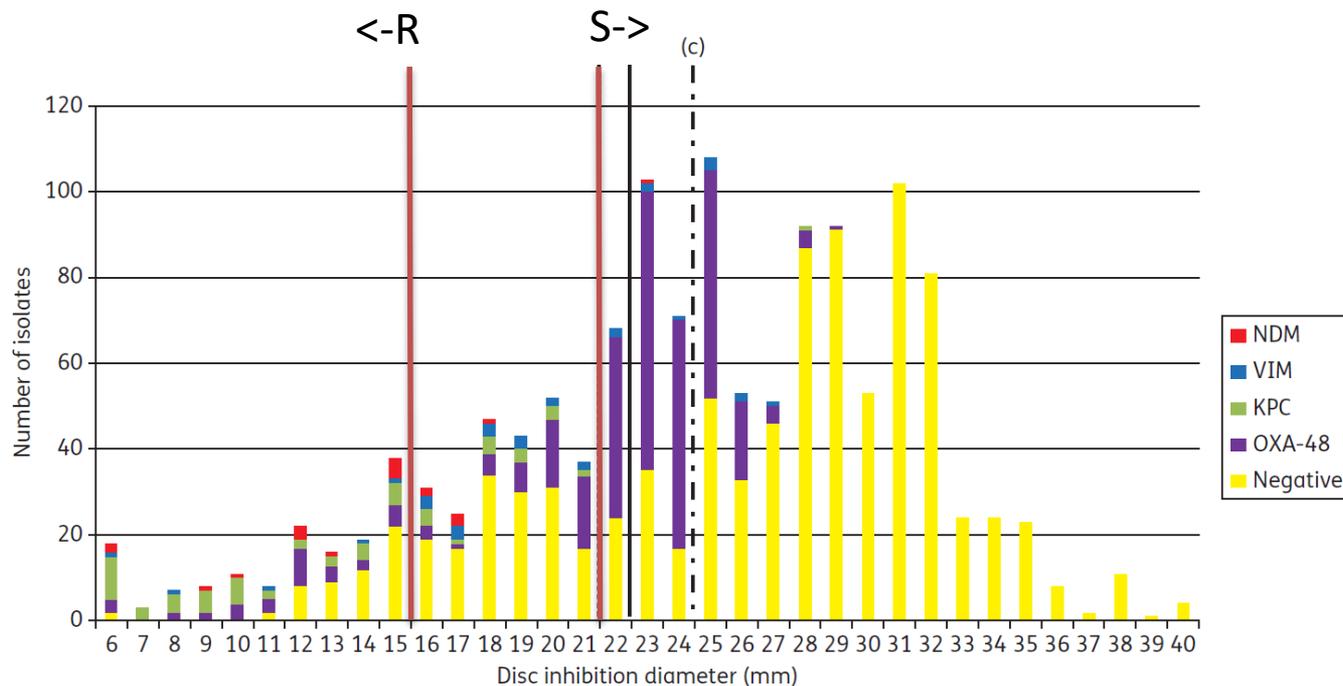
- KPC, IMI, NMC, GES-variants, FRI, NDM, VIM, IMP, GIM, OXA-48-variants +++++

Ambler class	Enzyme	β-lactam group						Inhibitor
		Penicillins	Early cephalosporins	Cephameycins	Extended-spectrum cephalosporins	Aztreonam	Carbapenems	
A	KPC	+	+	+/-	+	+	+	Avibactam, (clav. acid, tazobactam), boronic acid
	IMI	+	+	-	-	+/-	+	
	GES	+	+	+/-	+	-	+	
	FRI	+	+	-	+	+	+	
B	NDM, VIM, IMP etc.	+	+	+	+	-	+	EDTA, DPA
D	OXA-48-lik	+	+	+/-	-	-	+	(Avibactam)

- Note: variability in carbapenemase activity!

# WHY SCREENING CUT-OFF?

- *The presence of a carbapenemase do not always lead to clinical resistance!*



**Figure 1.** Distribution of meropenem 10 µg disc inhibition zone and of carbapenemases for Enterobacteriaceae isolates referred to the NRLs in 2012 (n=1354). (a) 2013 CLSI meropenem disc diffusion susceptibility zone diameter breakpoint ( $\geq 23$  mm). (b) 2013 EUCAST meropenem susceptibility zone diameter breakpoint ( $\geq 22$  mm). (c) 2013 EUCAST meropenem disc diffusion screening cut-off for the detection of CPE ( $< 25$  mm).



# MEROPENEM SCREENING CUT-OFF

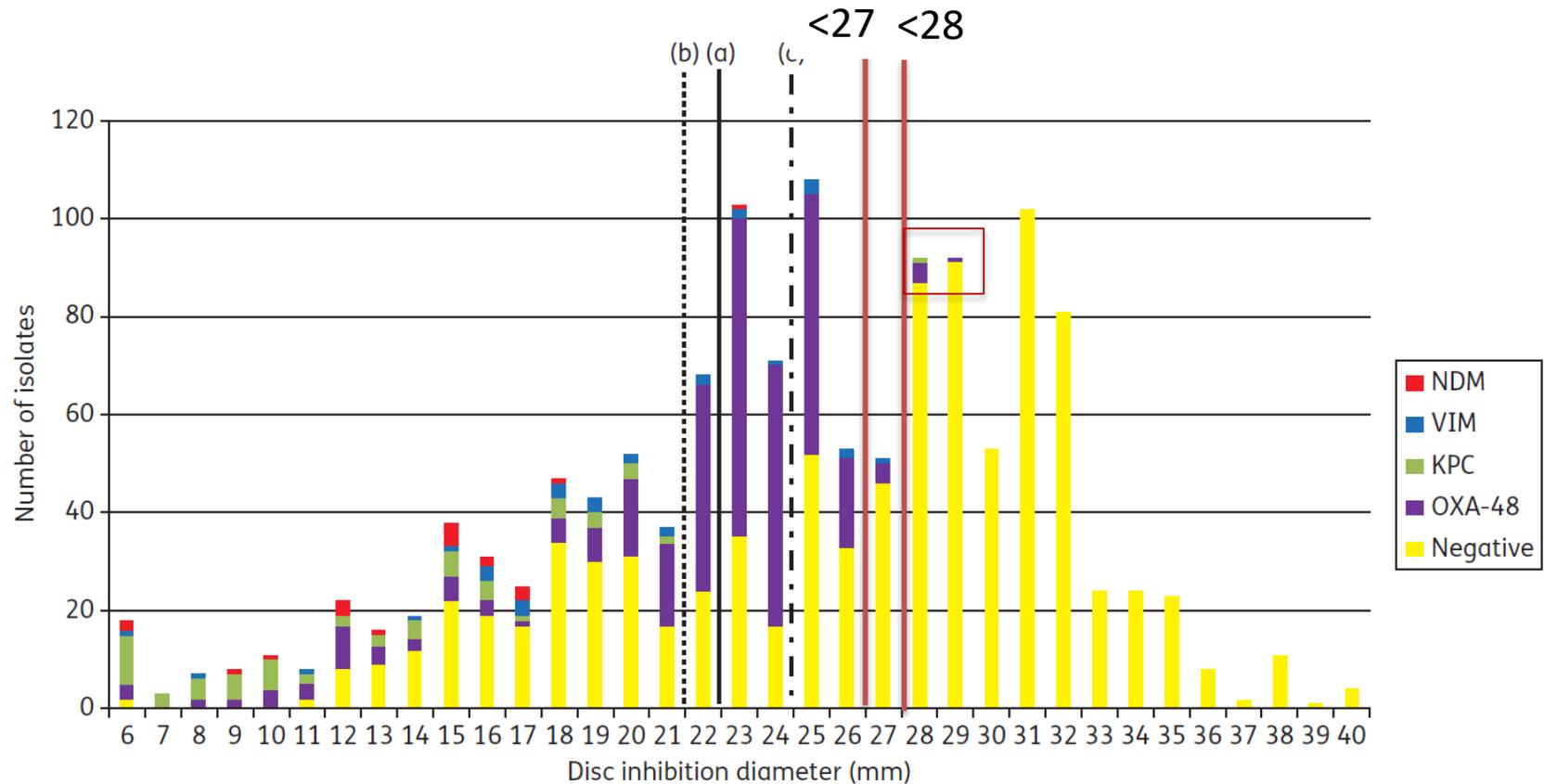
## ➤ 2017:

- Meropenem <27 mm (disc diffusion)/MIC > 0.125 mg/L
- Exception: meropenem 25-26 mm & pip-tazo = I/S – no further testing

## ➤ 2018:

- Meropenem <28 mm (disc diffusion)/MIC > 0.125 mg/L
- Exception: meropenem 25-27 mm & pip-tazo = I/S – no further testing

# IMPACT OF 1 mm



**Figure 1.** Distribution of meropenem 10 µg disc inhibition zone and of carbapenemases for Enterobacteriaceae isolates referred to the NRLs in 2012 (n=1354). (a) 2013 CLSI meropenem disc diffusion susceptibility zone diameter breakpoint (≥23 mm). (b) 2013 EUCAST meropenem susceptibility zone diameter breakpoint (≥22 mm). (c) 2013 EUCAST meropenem disc diffusion screening cut-off for the detection of CPE (<25 mm).

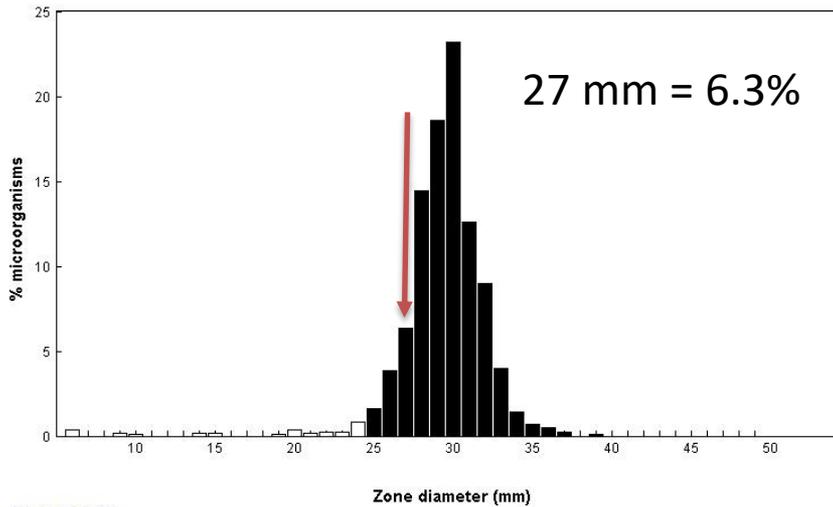
# IMPACT OF 1 mm?

- Identify more CPE
- Increased work load -> more isolates to investigate further
- Open questions:
  - How many more true CPE will we identify?
  - How large will the extra work be?
  - Same impact on all *Enterobacterales*?
  - Impact on the health care system?



**Meropenem / *Klebsiella pneumoniae***  
**International wild type zone diameter distribution - Reference database 2018-04-18**  
**EUCAST disk diffusion method**

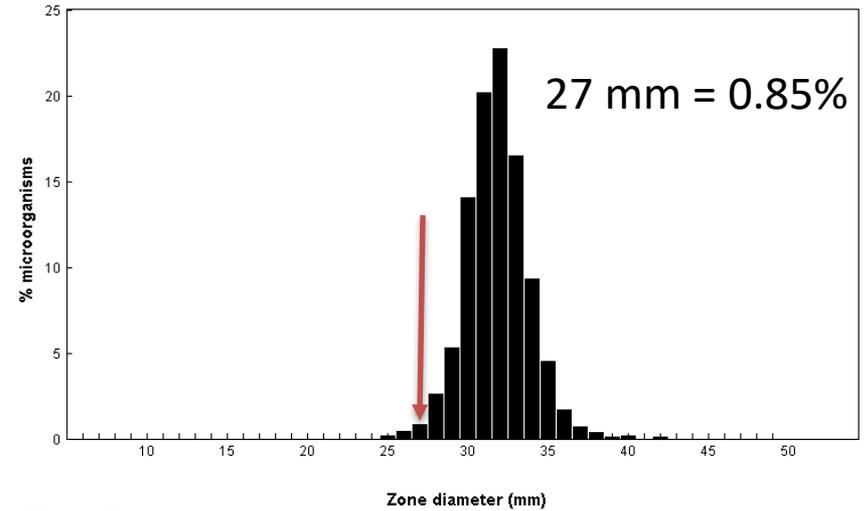
Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



Disk content: 10  
 Epidemiological cut-off (ECOFF): 25 mm (MIC = 0.125 mg/L)  
 Wildtype (WT) organisms:  $\geq 25$  mm (MIC = 0.125 mg/L) 1671 observations (10 data sources)

**Meropenem / *Escherichia coli***  
**International wild type zone diameter distribution - Reference database 2018-04-18**  
**EUCAST disk diffusion method**

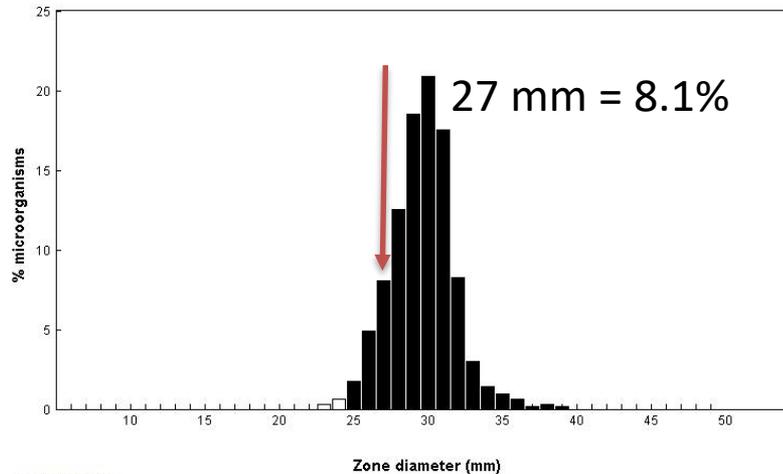
Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



Disk content: 10  
 Epidemiological cut-off (ECOFF): 25 mm (MIC = 0.125 mg/L)  
 Wildtype (WT) organisms:  $\geq 25$  mm (MIC = 0.125 mg/L) 11131 observations (14 data sources)

**Meropenem / *Enterobacter cloacae***  
**International wild type zone diameter distribution - Reference database 2018-04-18**  
**EUCAST disk diffusion method**

Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



Disk content: 10  
 Epidemiological cut-off (ECOFF): 25 mm (MIC = 0.125 mg/L)  
 Wildtype (WT) organisms:  $\geq 25$  mm (MIC = 0.125 mg/L) 632 observations (7 data sources)

➤ ECOFF:  $\geq 25$  mm

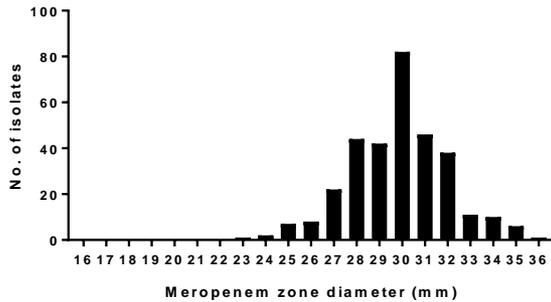
➤ Impact – species dependent



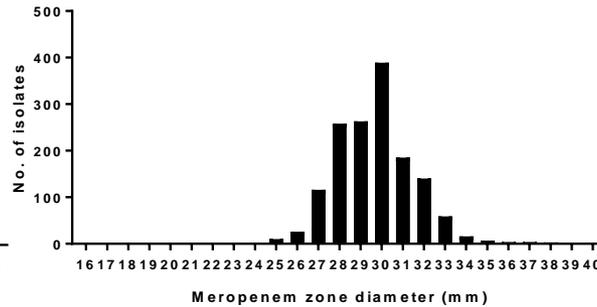
# PLAYING WITH NUMBERS



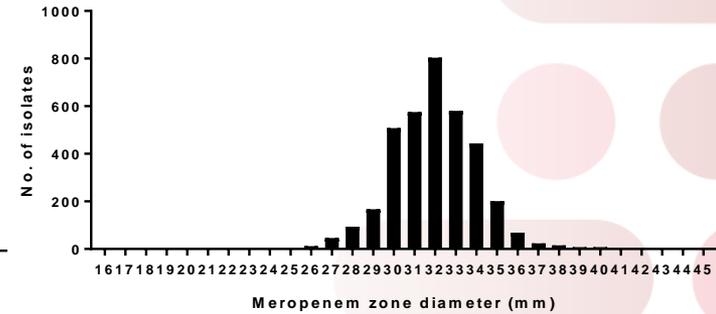
*Enterobacter* spp. blood  
NORM 2016 (n=320)



*K. pneumoniae* blood & urine  
NORM 2016 (n=1484)



*E. coli* blood & urine -  
NORM 2016 (n=3561)



Species	No. of isolates 27 mm	% of total	% TZP R	Extra isolates to investigate/year <sup>b</sup>
<i>Enterobacter</i> spp.	22	6.9%	<b>36%</b>	75
<i>K. pneumoniae</i>	116	7.8%	5.2%	62
<i>E. coli</i>	46 <sup>a</sup>	1.3% <sup>a</sup>	3.3% <sup>a</sup>	88

<sup>a</sup>based on blood culture isolates. <sup>b</sup>Calculated according to sampling period etc.

**A minimum of ~225 isolates extra from blood and urine to be analysed in Norway/year**



# IMPACT FOR THE CLINICAL LAB

Species	Tot. no. of isolates (2017)	Meropenem zone diameter = 27 mm	
		No. of isolates (% of total)	Pip-tazo R (% of 27 mm)
<i>C. freundii</i> cmplx	477	29 (6.1%)	3 (10.3%)
<i>C. koseri</i>	583	21 (3.6%)	
<i>E. aerogenes</i>	215	16 (7.4%)	2 (12.5%)
<i>E. cloacae</i> cmplx	839	52 (6.2%)	8 (15.4%)
<i>E. coli</i>	18638	342 (1.8%)	10 (2.9%)
<i>K. oxytoca</i>	889	27 (3.0%)	2 (7.4%)
<i>K. pneumoniae</i>	2795	218 (7.8%)	21 (9.6%)
<i>M. morgani</i>	264	12 (4.5%)	
<i>P. mirabilis</i>	949	79 (8.3%)	
<i>P. vulgaris</i>	383	24 (6.3%)	
<i>S. marescens</i>	245	19 (7.8%)	1 (5.3%)
<b>Total</b>	<b>26277</b>	<b>839 (3.2%)</b>	<b>47 isolates</b>

➤ KMA Skejby: 115 isolates; KMA Odense: 10 isolates



# IMPACT ON THE HOSPITAL & PATIENT

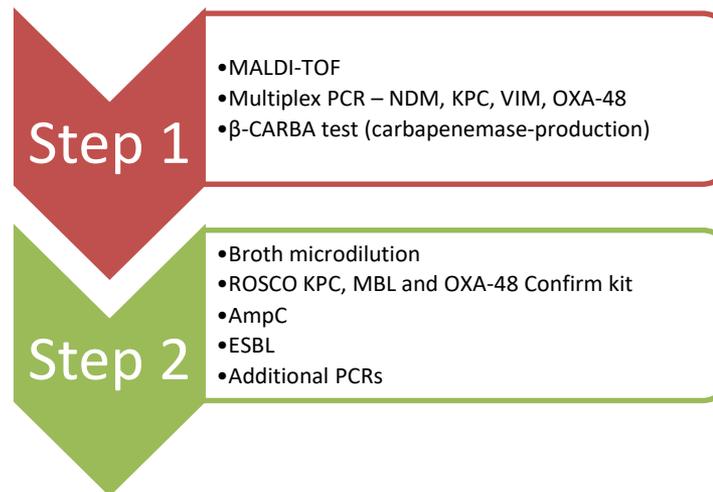
Importance of detection of resistance mechanism	
Required for <b>clinical</b> antimicrobial susceptibility categorization	No
Infection control <b>purposes</b>	Yes
Public health <b>purposes</b>	Yes

- Infection control measures
  - Isolation of patient etc
  - Contact precatution
  - Screening other patients – outbreak investigation
  
- Increased down-stream work for verification of carbapenemase-production
  
- Submit isolate to reference laboratory



# IMPACT ON REFERENCE LAB

- Norway: ~225 isolates extra/year
  - 2017: 183 *Enterobacterales* analysed for carbapenemase-production
- 
- Should we «do everything» on all suspected CPE?
  - What is the predictive value of the CPE tests?
  - Should we add extra criteria for submission of isolates?



# **Enterobacter – Ref. lab Norway**

Year	Criteria for submission	No. of isolates
2014	<25 mm	11
2015	<27 mm*	42
2016	<27 mm*	52
2017	<27 mm*	77
Total		182

\* Meropenem 25-26 mm & TZP-S -> no further testing

- Do the labs follow the submission criteria?
- Do disc diffusion and MIC correlate? MIC screening bp. not changed
- How many of these were carbapenemase-producers?
- Can phenotypic/biochemical tests predict carbapenemase-production?



# Should isolates be analysed?

- Disc diffusion & broth microdilution – meropenem

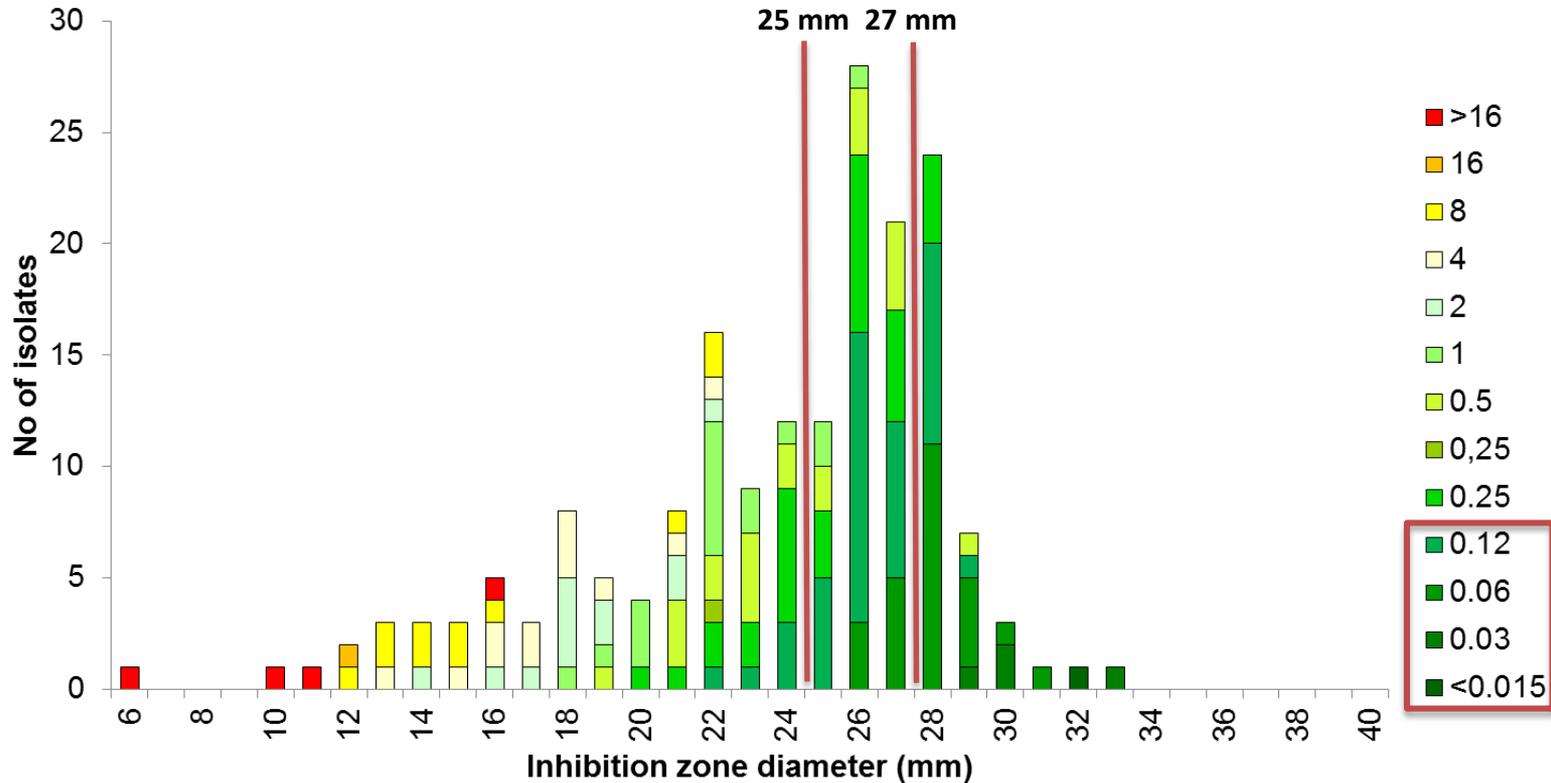
MIC	Zone diameter			
	> 27 mm	25-27 mm + TZP S/I	25-27 mm + TZP R	< 25 mm
≤ 0.12	<b>32</b>	<b>7</b>	26	5
0.25	4	0	16	13
0.5	1	0	9	12
≥ 1	0	1	2	54

- 21.4% - does not fulfil the screening criteria (both MIC & DD)
- 17.0% - should be analysed based on disc diffusion, but not based on MIC



# CORRELATION ZONE DIAMETER/MIC

## Meropenem 10 µg vs. MIC



➤ Disk diffusion more «sensitive»

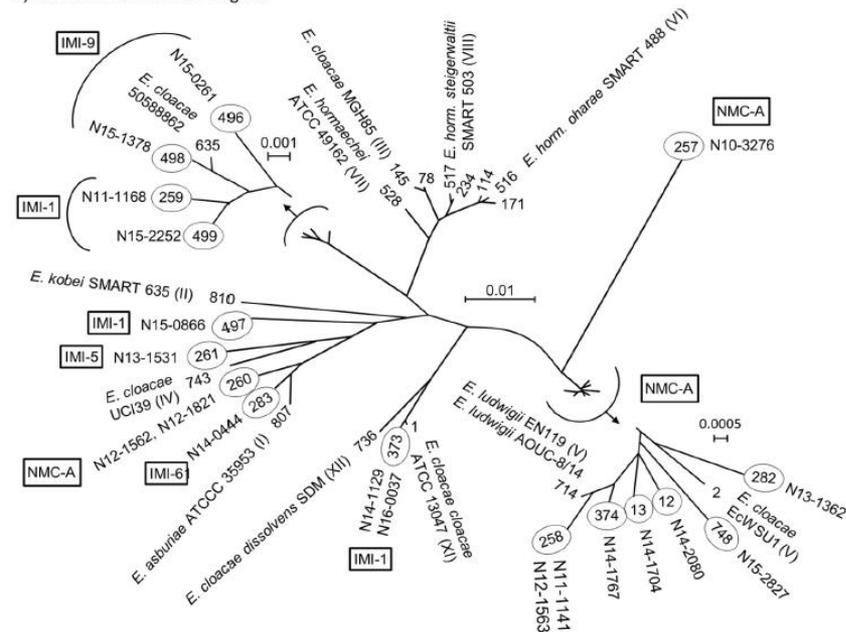
# CARBAPENEMASES?

➤ PCR: KPC, VIM, NDM, OXA-48-like + NMC & IMI

➤ NMC/IMI

- Class A carbapenemases – mainly identified in *Enterobacter*

A) concatenated MLST targets



β-Lactam	
ECC 50588862	
Ampicillin <sup>a</sup>	>256
Amoxicillin/clavulanic acid	>128
Piperacillin <sup>a</sup>	8
Piperacillin/tazobactam	2
Ceftazidime	0.5
Cefotaxime	0.25
Cefuroxime	8
Cefoxitin	>32
Aztreonam	0.5
Meropenem	2
Imipenem	16
Ertapenem	4



# CARBAPENEMASES?

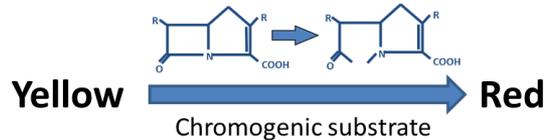
Year	Species	Carbapenemase	MEM MIC (mg/L)	MEM zone (mm)	Import
<b>2014</b>	<i>E. cloacae</i> cmplx	KPC	> 16	6	Jamaica
	<i>E. cloacae</i> cmplx	NDM	> 16	16	No
<b>2016</b>	<i>E. cloacae</i> cmplx	OXA-48-like	0.5	23	Brazil
	<i>E. cloacae</i> cmplx	VIM	1	22	Croatia
	<i>E. cloacae</i> cmplx	NMC-A	8	13	Spain
<b>2017</b>	<i>E. cloacae</i> cmplx	NDM	2	22	Serbia
	<i>E. cloacae</i> cmplx	NDM	8	21	Marocco

➤ 7/142 (4.9%) carbapenemase positive

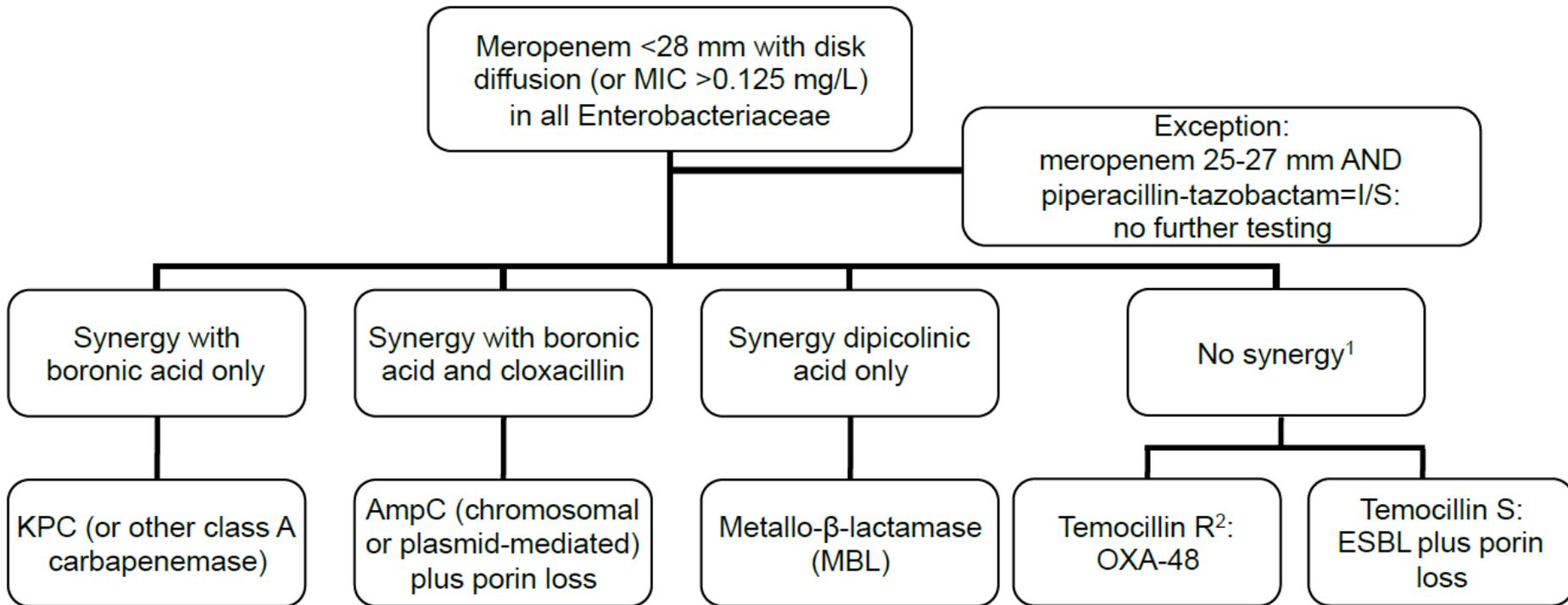
➤ Canada 2010-2015: 6.04% (Boyd DA *et al. Antimicrob. Agents Chemother.* 2017)

# PHENOTYPIC CHARACTERISTICS

- $\beta$ -CARBA test



- ROSCO KPC, MBL and OXA-48 Confirm kit





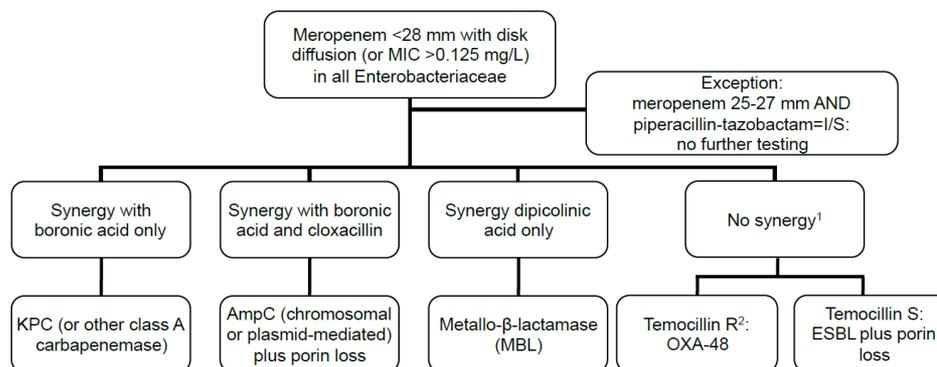
# β-CARBA TEST

Year	Species	Carbapenemase	MEM MIC (mg/L)	MEM zone (mm)	β-CARBA
2014	<i>E. cloacae</i> cmplx	KPC	> 16	6	+
	<i>E. cloacae</i> cmplx	NDM	> 16	16	+
2016	<i>E. cloacae</i> cmplx	OXA-48-like	0.5	23	+
	<i>E. cloacae</i> cmplx	VIM	1	22	+
	<i>E. cloacae</i> cmplx	NMC-A	8	13	-
2017	<i>E. cloacae</i> cmplx	NDM	2	22	+
	<i>E. cloacae</i> cmplx	NDM	8	21	+

- 6/7 carbapenemase-producers positive with β-CARBA test
- No false positive test result
- Specificity: 100%; Sensitivity: 86%; PPV: 100%; NPV: 99%



# ROSCO KPC, MBL and OXA-48 Confirm kit



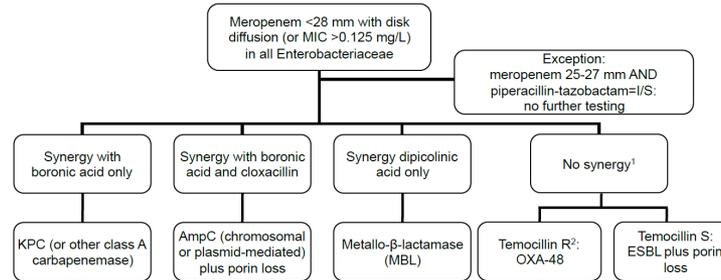
Year	Species	Carbapenemase	MEM MIC (mg/L)	MEM zone (mm)	ROSCO
2014	<i>E. cloacae</i> cmplx	KPC	> 16	6	KPC (class A)
	<i>E. cloacae</i> cmplx	NDM	> 16	16	MBL
2016	<i>E. cloacae</i> cmplx	OXA-48-like	0.5	23	No syn, TMO R -> OXA-48
	<i>E. cloacae</i> cmplx	VIM	1	22	MBL
	<i>E. cloacae</i> cmplx	NMC-A	8	13	KPC (class A)
2017	<i>E. cloacae</i> cmplx	NDM	2	22	MBL
	<i>E. cloacae</i> cmplx	NDM	8	21	MBL

➤ All carbapenemase-producers positive and correctly grouped



# ROSCO KPC, MBL and OXA-48 Confirm kit

## CARBAPENEMASE-NEGATIVE ISOLATES



	<b>KPC (class A)</b>	<b>AmpC + porin loss</b>	<b>MBL</b>	<b>OXA-48</b>	<b>Negative</b>
<b>Carbapenemase-negative (n=135)</b>	<b>13</b>	40	0	<b>3</b>	79

- 9.6% false positive KPC (class A)
- 2.2% false positive OXA-48
- Specificity: 88%; Sensitivity: 100%; PPV: 30%; NPV: 100%



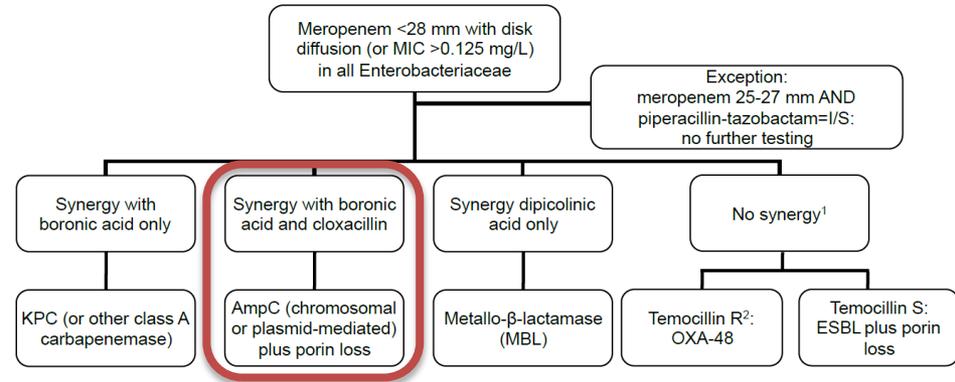
# MECHANISM OF CARBAPENEM RESISTANCE?

AMERICAN SOCIETY FOR MICROBIOLOGY Antimicrobial Agents and Chemotherapy



Association of Novel Nonsynonymous Single Nucleotide Polymorphisms in *ampD* with Cephalosporin Resistance and Phylogenetic Variations in *ampC*, *ampR*, *ompF*, and *ompC* in *Enterobacter cloacae* Isolates That Are Highly Resistant to Carbapenems

Baharak Babouee Flury,<sup>a</sup> Matthew J. Ellington,<sup>a</sup> Katie L. Hopkins,<sup>a</sup> Jane F. Turton,<sup>a</sup> Michel Doumith,<sup>a</sup> Richard Loy,<sup>a</sup> Peter Staves,<sup>a</sup> Vladimira Hincic,<sup>a</sup> Reno Frei,<sup>a</sup> Neil Woodford<sup>a</sup>  
Antimicrobial Resistance and Healthcare Associated Infections (AMRHA) Reference Unit, Public Health England, London, United Kingdom<sup>a</sup>; Division of Clinical Microbiology, University Hospital Basel, Basel, Switzerland<sup>a</sup>

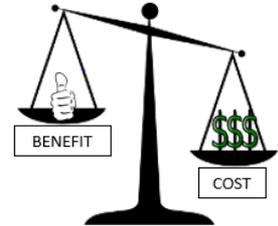


- **KPC, MBL and OXA-48 Confirm kit:**
  - 30% positive for AmpC + porin loss
- **ROSCO AmpC Kit:**
  - 97% positive for AmpC
  - All carbapenemase-positive (except OXA-48-positive isolate) negative for AmpC
- ESBL + porin loss?

# CONCLUSIONS

➤ 1 mm makes a lot of difference!

- Cost/benefit?
  - None of the isolates with 27 mm in NORM 2016 were CPE.
- Should all *Enterobacterales* have the same screening breakpoint?
  - *Enterobacter* spp.: 25 mm?
- Additional screening criteria?
  - Temocillin? IMI/NMC-A isolates susceptible to temocillin!





# ACKNOWLEDGEMENT

- **Bjørg Haldorsen**
- **Erika Matuschek**
- **Barbara Holzknecht, Ulrik Justesen, Mikala Wang**

*Thank you for your attention!*