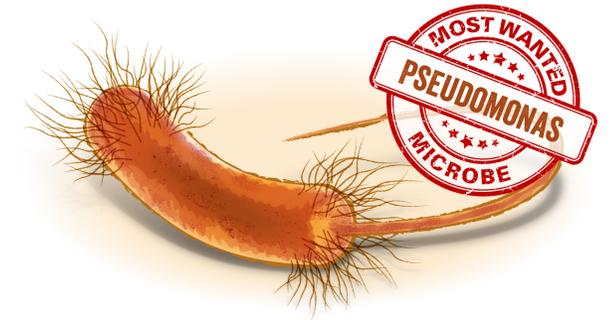


# Interpretation of resistance mechanisms of *Pseudomonas aeruginosa* and/or *Acinetobacter* susceptibility testing

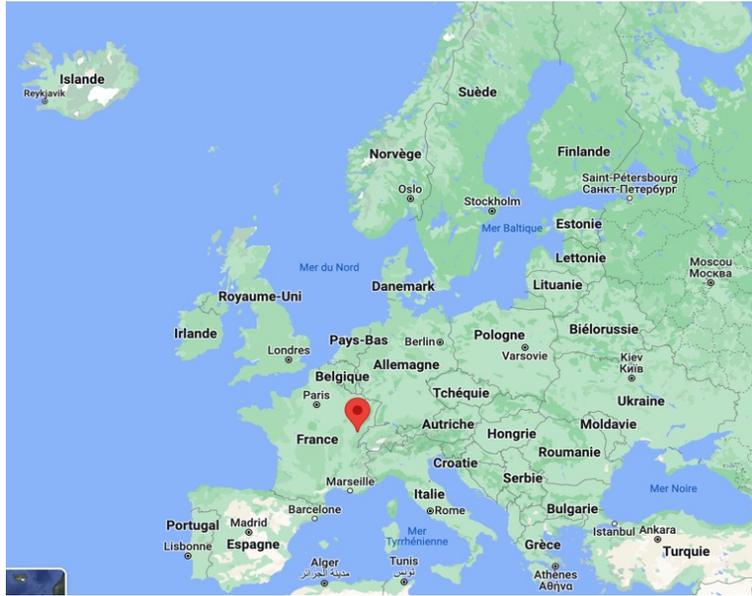
Katy Jeannot

Laboratory of Bacteriology, University hospital of Besançon  
National Reference Center for Antibiotic resistance  
« *Pseudomonas* spp, *Acinetobacter* spp »



# University hospital of Besançon, France

Besançon  
(France)



## NRC group

- Damien Fournier
- Anaïs Potron
- Xavier Vuillemin
- Maxime Bour
- Pauline Triponney
- Emma Gauthier



University hospital (1,500-beds)

# Wild-type drug susceptibility of *P. aeruginosa*

---

## $\beta$ -lactams

**Piperacillin**

**Piperacillin + tazobactam**

Aztreonam

**Ceftazidime**

*Ceftazidime + avibactam*

**Cefepime**

Ceftobiprole

*Ceftolozane + tazobactam*

*Cefiderocol*

**Imipenem**

*Imipenem + relebactam*

**Meropenem**

*Meropenem + vaborbactam*

Doripenem

## Aminoglycosides

**Tobramycin**

**Amikacin**

## Fluoroquinolones

**Ciprofloxacin**

Levofloxacin

Delafloxacin

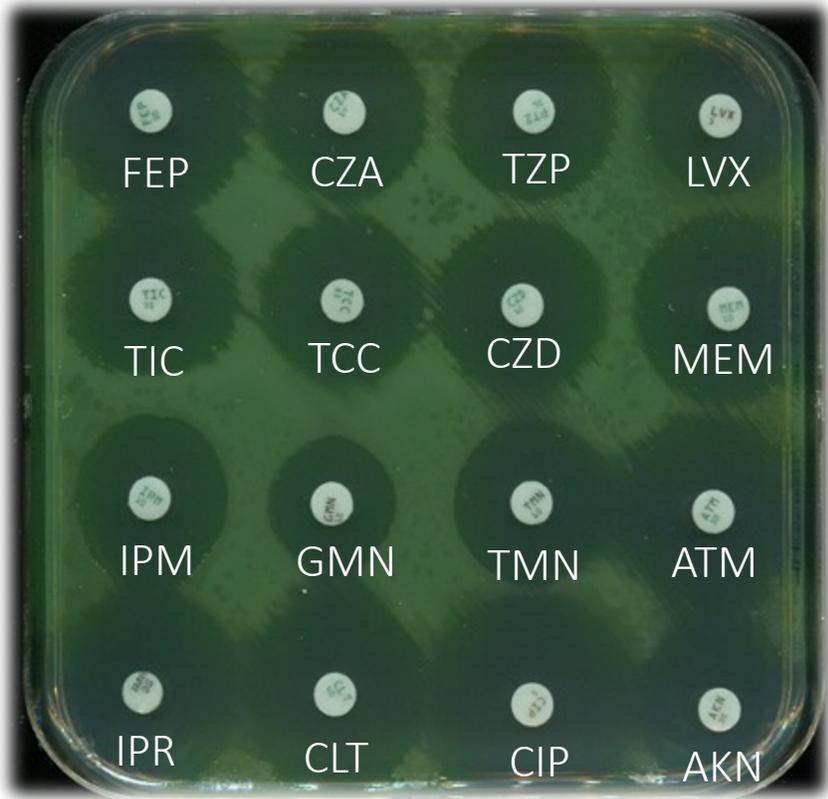
## Others

Colistin

Polymyxin B

Fosfomycin IV

# Intrinsic resistance of *P. aeruginosa*



FEP (cefepime), CZA (ceftazidime/avibactam), TZP (piperacillin-tazobactam), LVX (levofloxacin), TIC (ticarcillin), TCC (ticarcillin-clavulanate), CZD (ceftazidime), MEM (meropenem), IPM (imipenem), GMN (gentamicin), TMN (tobramycin), ATM (aztreonam), IPR (imipenem/relebactam), CLT, (ceftolozane/tazobactam), CIP (ciprofloxacin), AKN (amikacin)

- ✓ Wide-spectrum inducible  $\beta$ -lactamase **AmpC** (aminopenicillins, 1<sup>st</sup>GC, 2<sup>nd</sup>GC)
- ✓ Narrow-spectrum penicillinase **OXA-50 (PoxB)** (Tic)
- ✓ Aminoglycoside-modifying enzyme **APH(3')-IIb** (Km, Neo)
- ✓ Poorly permeable **porin pathway (OprF)** (all antibiotics except polymyxins, aminoglycosides, carbapenems)
- ✓ Active efflux system **MexAB-OprM** ( $\beta$ -lactams except Ipm, macrolides, tetracyclines, fluoroquinolones, cefotaxime, ertapenem)
- ✓ Active efflux system **MexXY/OprM** (aminoglycosides, macrolides, tetracyclines)
- ✓ Fosfomycin modifying enzyme **FosA** (glutathione S-transferase)

# Active antibiotics in *A. baumannii*

---

## $\beta$ -lactams

- ✓ Ampicillin-sulbactam
- ✓ Piperacillin
- ✓ Ceftazidime
- ✓ Cefepim
- ✓ *Cefiderocol*
- ✓ Imipenem
- ✓ Meropenem
- ✓ Doripenem



Ceftolozane /tazobactam: CMI<sub>50</sub> >16, CMI<sub>90</sub> >32 mg/L  
Ceftazidime/avibactam: CMI<sub>50</sub> >16, CMI<sub>90</sub> >32 mg/L  
Ceftobiprole CMI<sub>50</sub> >1, CMI<sub>90</sub> =32 mg/L  
Imipenem/relebactam and Meropenem/vaborbactam

## Aminoglycosides

- ✓ Tobramycin
- ✓ Amikacin
- ✓ Gentamicin

## Fluoroquinolones

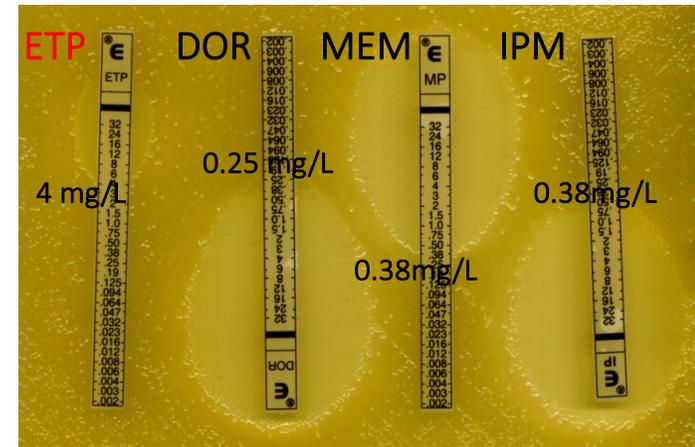
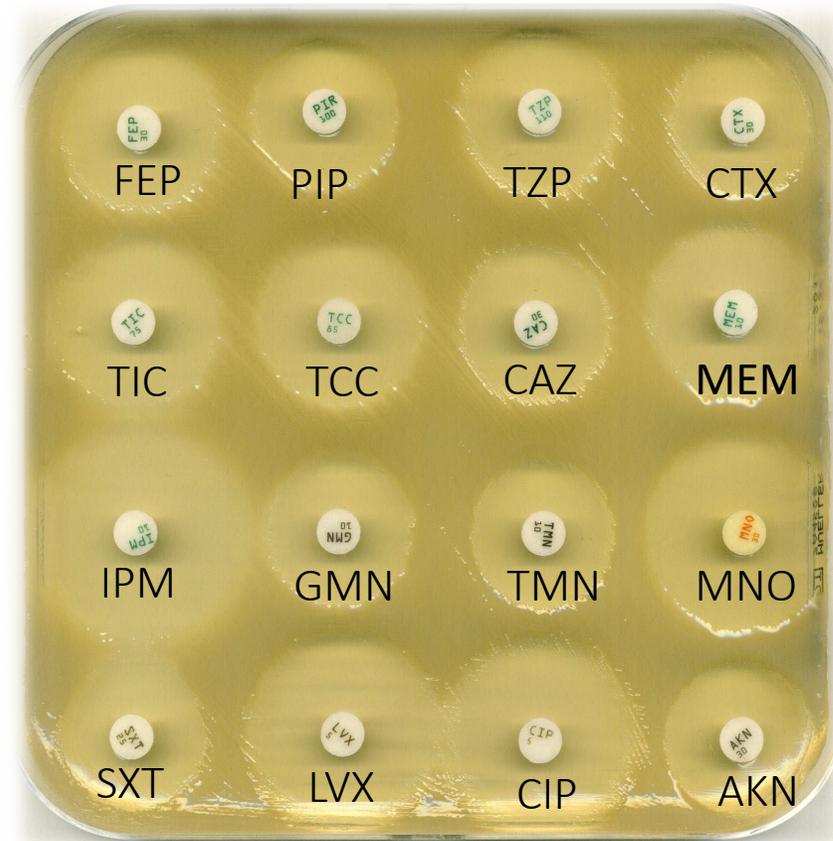
- ✓ Ciprofloxacin
- ✓ Levofloxacin

## Other

- ✓ Colistin, polymyxin B
- ✓ Trimethoprim/sulfamethoxazol
- ✓ Minocycline
- ✓ Tigecycline

# Wild-type strain, *A. baumannii*

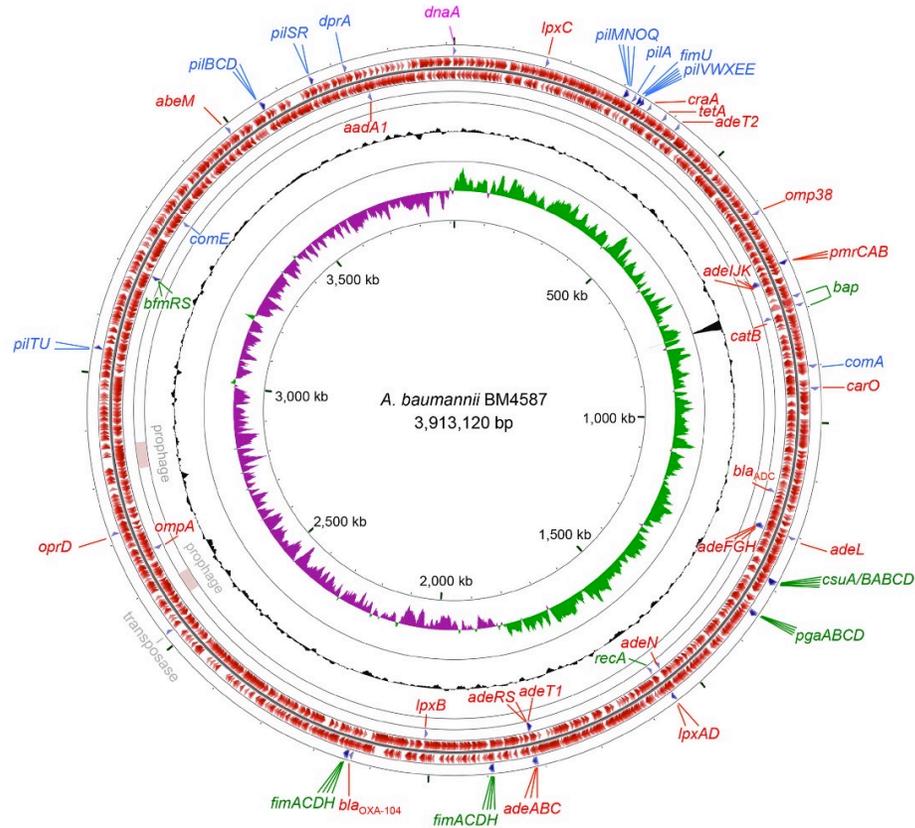
Mueller-Hinton Agar  
Disc diffusion



ETP (ertapenem), DOR (doripenem), MEM (meropenem), IPM, (imipenem)

FEP (cefepime), PIP (piperacillin), TZP (piperacillin-tazobactam), CTX (cefotaxime), TIC (ticarcillin), TCC (ticarcillin-clavulanate), CAZ (ceftazidime), MEM (meropenem), IPM (imipenem), GMN (gentamicin), TOB (tobramycin), MNO (minocyclin), SXT (trimethoprim/sulfamethoxazol), LVX (levofloxacin), CIP (ciprofloxacin), AKN (amikacin)

# Intrinsic resistance of *Acinetobacter baumannii*



Yoon E et al. 2015. *mBio* 6(2):e00309-15.

- ✓ Wide-spectrum  $\beta$ -lactamase **ADC** (aminopenicillins, 1<sup>st</sup>GC, 2<sup>nd</sup>GC)
- ✓ Oxacillinase, **OXA-51** and its variants (aminopenicillins, carboxypenicillin, ureidopenicillin)
- ✓ Poorly permeable
- ✓ Active efflux system **AdeJFK** ( $\beta$ -lactams except lpm, macrolides, tetracyclines, fluoroquinolones, phenicols)

# EUCAST and CLSI clinical breakpoints

e.g. *Pseudomonas aeruginosa*

	2023	
	EUCAST ( S ≤; > R)	CLSI ( S ≤; ≥R)
Ticarcillin / clavulanic acid	I ≤ 16 / 2	S ≤ 16 / 2 ; R ≥ 128 / 2
Piperacillin	I ≤ 16	S ≤ 16 ; R ≥ 64
Piperacillin / tazobactam	I ≤ 16 / 4	S ≤ 16 / 4 ; R ≥ 64 / 4
Ceftazidime	I ≤ 8	S ≤ 8 ; R ≥ 32
Cefepime	I ≤ 8	S ≤ 8 ; R ≥ 32
Ceftazidime / avibactam	S ≤ 8 / 4	S ≤ 8 / 4 ; R ≥ 16/4
Ceftolozane / tazobactam	S ≤ 4 / 4	S ≤ 4 / 4 ; R ≥ 16/ 4
Cefiderocol	S ≤ 2	S ≤ 4 ; R ≥ 16
Imipenem	I ≤ 4	S ≤ 2 ; R ≥ 8
Imipenem / relebactam	S ≤ 2	S ≤ 2/4 ; R ≥ 8
Meropenem	S ≤ 2 ; R > 8	S ≤ 2 ; R ≥ 8
Meropenem (meningitidis)	S ≤ 2 ; R > 2	-
Meropenem/vaborbactam	S ≤ 8 / 8	-
Doripenem	I ≤ 2	S ≤ 2 ; R ≥ 8
Aztreonam	I ≤ 16	S ≤ 8 ; R ≥ 32
Ciprofloxacin	I ≤ 0.5	S ≤ 0.5 ; R ≥ 2
Levofloxacin	I ≤ 2	S ≤ 1 ; R ≥ 4
Amikacin (urinary infection)	S ≤ 16	S ≤ 16 ; R ≥ 64
Amikacin	(≤ 16)	-
Tobramycin (urinary infection)	S ≤ 2	S ≤ 1 ; R ≥ 4
Tobramycin	(≤ 2)	-
Colistin	(≤ 4)	S ≤ 2 ; R ≥ 4

**S, Susceptible** standard dosing regimen

**I, Susceptible increased** exposure

**R, Resistant**



- Interpretation of susceptibility testing
- Interpretation of epidemiological data on resistance

# Antibiogram Interpretation of a wild-type strain of *P. aeruginosa*

Antibiotics	S ≤	(mg/L)	R >	Interpretation EUCAST 2023
Ticarcillin/clavulanic acid	0.001		16	I
Piperacillin/tazobactam	0.001		16	I
Ceftazidime	0.001		8	I
Cefepime	0.001		8	I
Imipenem	0.001		4	I
Meropenem*	2		8	S
Ciprofloxacin	0.001		0.5	I
Levofloxacin	0.001		2	I
Amikacin	(16)		(16)	S <sup>a</sup> /-
Tobramycin	(2)		(2)	S <sup>a</sup> /-
Colistin	(4)		(4)	-



\* Other than meningitidis, <sup>a</sup> a urinary tract infection, (-) BP with and without acquired resistance mechanisms

Wild type : without phenotypically detectable resistance mechanisms

I: Susceptible increased exposure

# Impact of EUCAST criteria in *P. aeruginosa*

- ★ Retrospective study
- ★ University hospital of Lausanne, Switzerland (1 000 lits)
- ★ Clinical samples positives to *P. aeruginosa* (August 2019-July 2020)
- ★ Isolates susceptible to ceftazidime, cefepime and/or piperacillin/tazobactam and meropenem

	<u>2019 (n=148)</u>	<u>2020 (n=116)</u>		
<b>Type of antibiotic prescribed after susceptibility testing</b>				
Cefepime	19 (12.8)	11 (9.5)	<b>n=264 patients</b>	
Ceftazidime	8 (5.4)	7 (6.0)		
Ciprofloxacin	30 (20.3)	19 (16.4)		
Imipenem	1 (0.7)	1 (0.9)		
Levofloxacin	4 (2.7)	1 (0.9)		
Meropenem	5 (3.4)	35 (30.2)		
Piperacillin-tazobactam	77 (52.0)	41 (35.3)		
Other	4 (2.7)	1 (0.9)		
<b>IDs consultation after susceptibility testing</b>				
None	88 (59.5)	43 (37.1)		
Supervision of first target therapy	33 (22.3)	54 (46.5)		
Follow-up IDs advice	60 (40.5)	73 (62.9)		
<b>Correct targeted antibiotic dosing</b>	76 (55.5)	72 (88.9)	<b>Muntig A et al. CMI, 2021</b>	
<b>Dosing adjustment of targeted antibiotic after ID advice</b>	41 (28.7)	55 (67.9)		

# Impact on $\beta$ -lactamase inhibitors in wild-type strain

Antibiotic	S ≤	(mg/L)	R >	EUCAST 2023	
Ticarcillin/clavulanic acid	0.001		16	I	
Piperacilline/tazobactam	0.001		16	I	
<b>Ceftazidime</b>	<b>0.001</b>		<b>8</b>	<b>I (2g x 3)</b>	
<b>Ceftazidime/avibactam</b>	<b>8</b>		<b>8</b>	<b>S (2 g + 0.5g x 3)</b>	
Ceftolozane/tazobactam	4		4	S	
Cefepime	0.001		8	I	
<b>Imipenem</b>	<b>0.001</b>		<b>4</b>	<b>I (1g x 4)</b>	
<b>Imipenem/relebactam</b>	<b>2</b>		<b>2</b>	<b>S (0.5g +0.25g X4)</b>	
<b>Meropenem</b>	<b>2</b>		<b>8</b>	<b>S (2g x 3)</b>	
<b>Meropenem/vaborbactam</b>	<b>8</b>		<b>8</b>	<b>S (2g +2g x3)</b>	
Cefiderocol	2		2	S	

# Antibiogramm interpretation of a wild-type strain of *Acinetobacter* spp

Antibiotics	S ≤	(mg/L)	R >	Interpretation <b>2023</b>
Doripenem	0.001		8	I
Imipenem	2		4	S
Meropenem*	2		8	S
Ciprofloxacin	0.001		1	I
Levofloxacin	0.5		1	S
Amikacin	8		8	S <sup>a</sup>
Tobramycin	4		4	S <sup>a</sup>
Gentamicin	4		4	S <sup>a</sup>
Trimethoprim-sulfamethoxazol	2		4	S
Colistin	(2)		(2) <sup>a</sup>	-

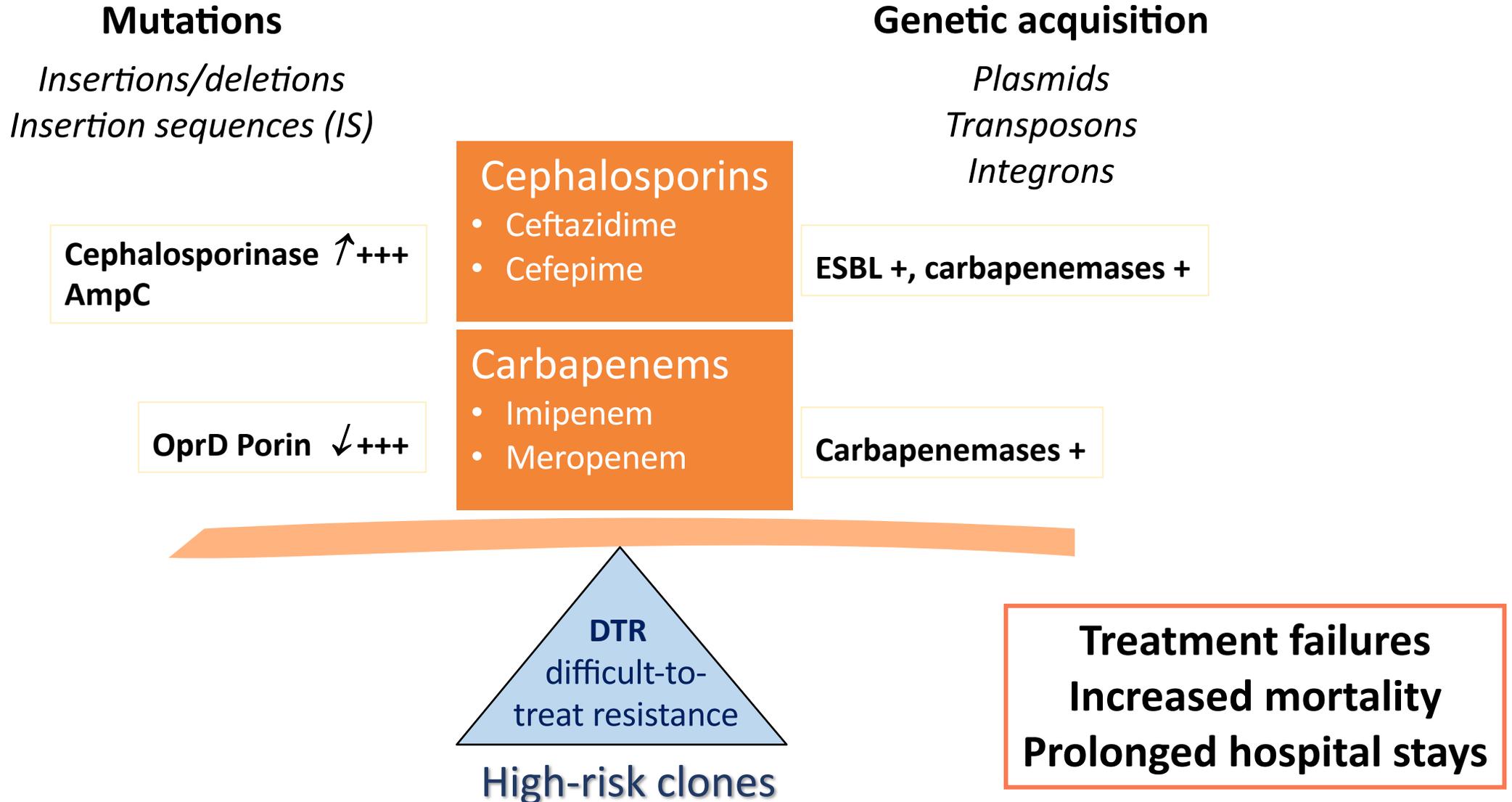
(-) BP with and without acquired resistance mechanisms

\* Other than meningitidis, <sup>a</sup>infection from the urinary tract

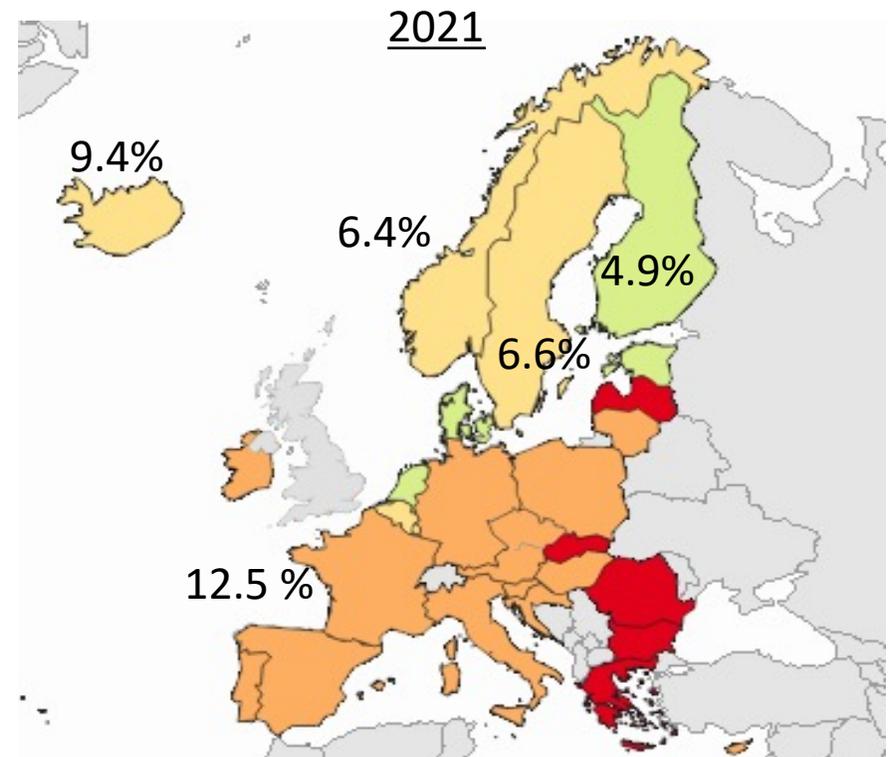
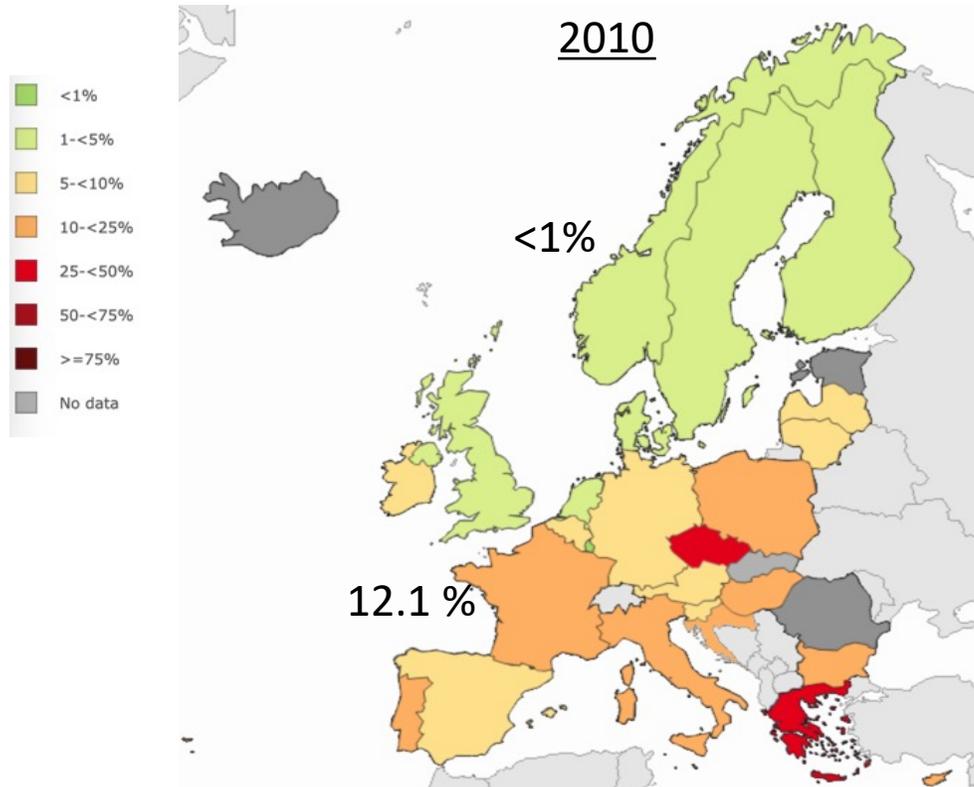
Wild type : without phenotypically detectable resistance mechanisms

I: Susceptible increased exposure

# Molecular mechanisms of drug resistance in *P. aeruginosa*

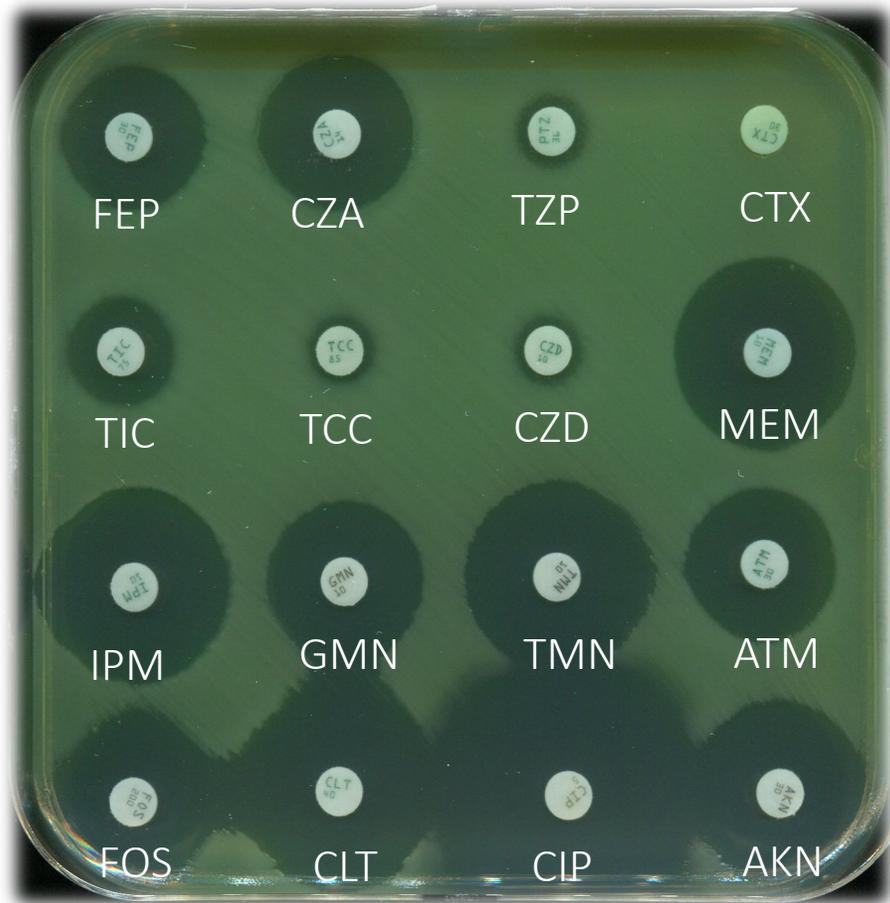


# Ceftazidime resistance in *P. aeruginosa* in Europe



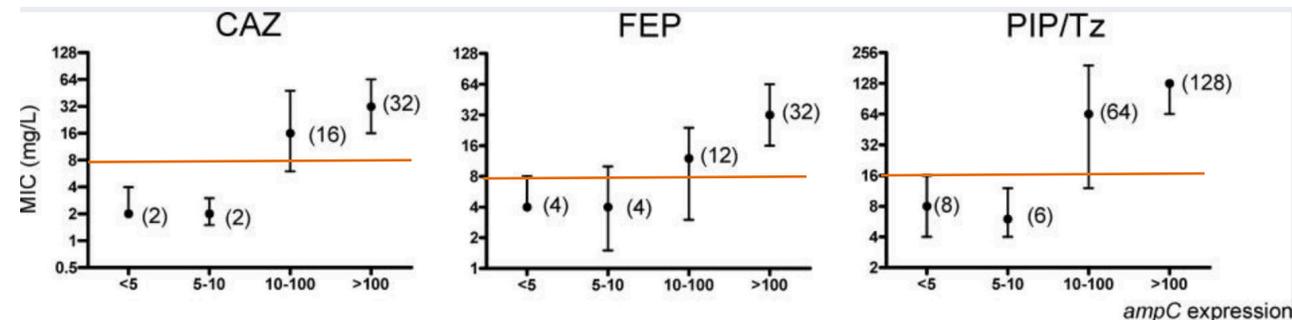
ECDC. Antimicrobial resistance in the EU/EEA (EARS-Net)

# Overproduction of AmpC, PDC cephalosporinase



Mueller-Hinton

The most frequent resistance mechanism to ceftazidime in clinical isolates

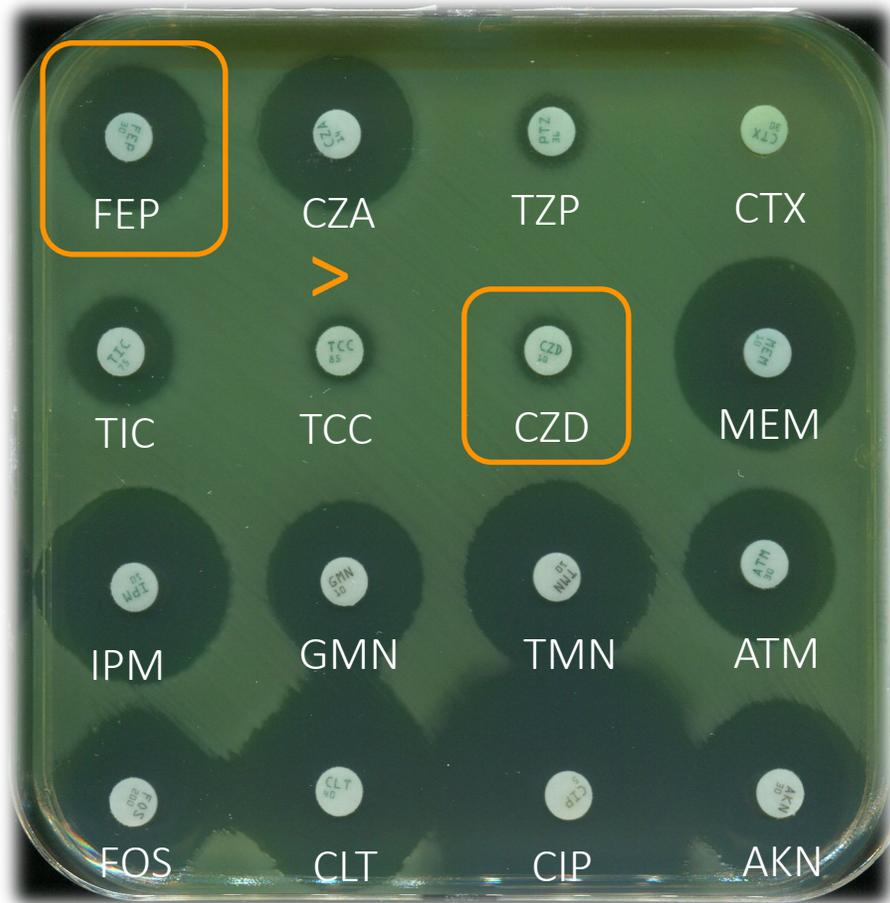


Cabot G et al., AAC, 2011

Variations in gene expression of *bla*<sub>AmpC</sub>

# Overproduction of AmpC, PDC cephalosporinase

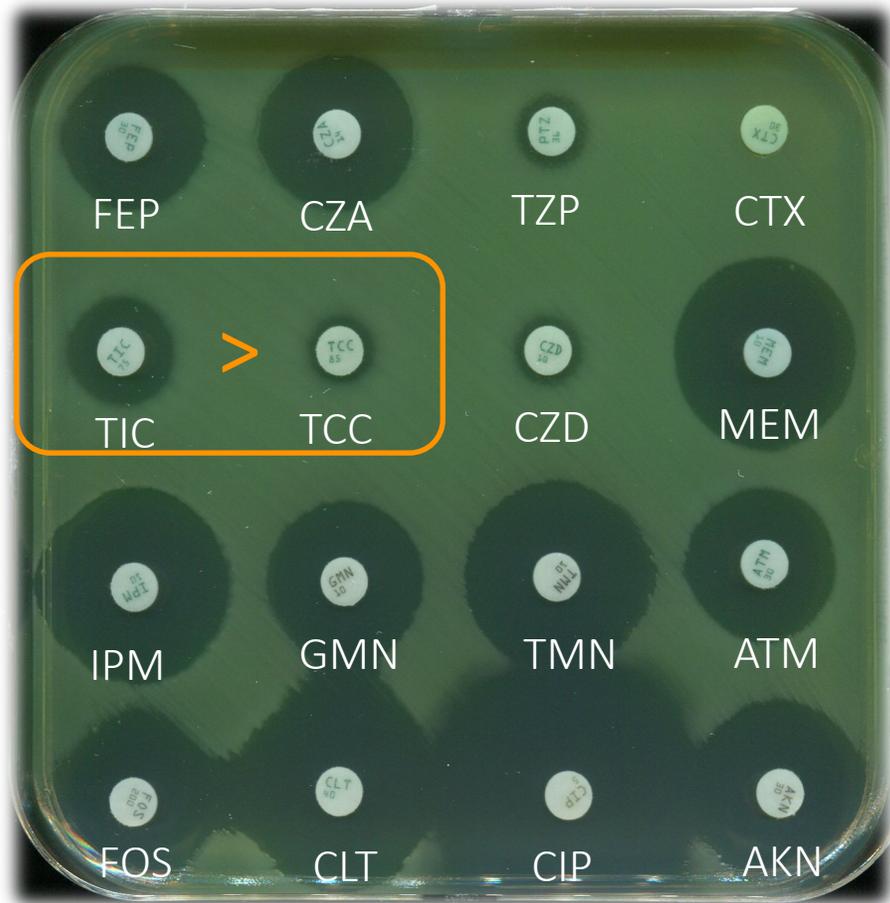
---



- ★ Inhibition zone diameter of cefepime is greater than that of ceftazidime

# Overproduction of AmpC, PDC cephalosporinase

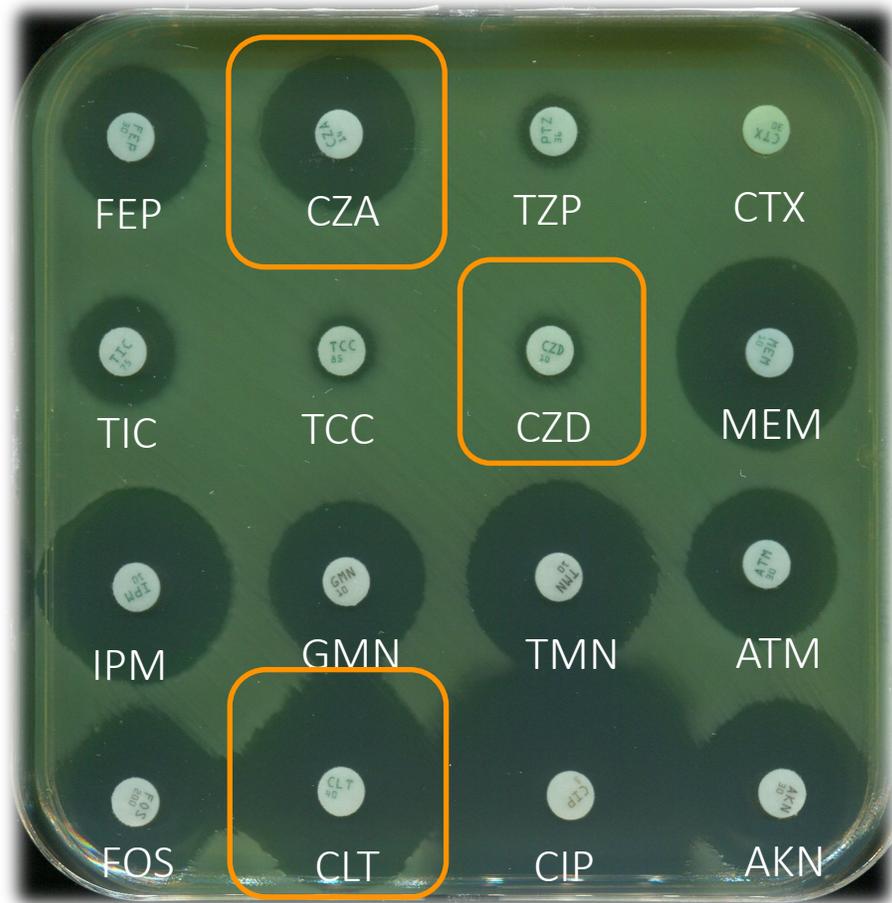
---



- ✦ Inhibition zone diameter of ticacillin/clavulanic acid is lower than that of ticarcillin
- ✦ Due to clavulanic acid induction of the *ampC* cephalosporinase expression

# Overproduction of AmpC, PDC cephalosporinase

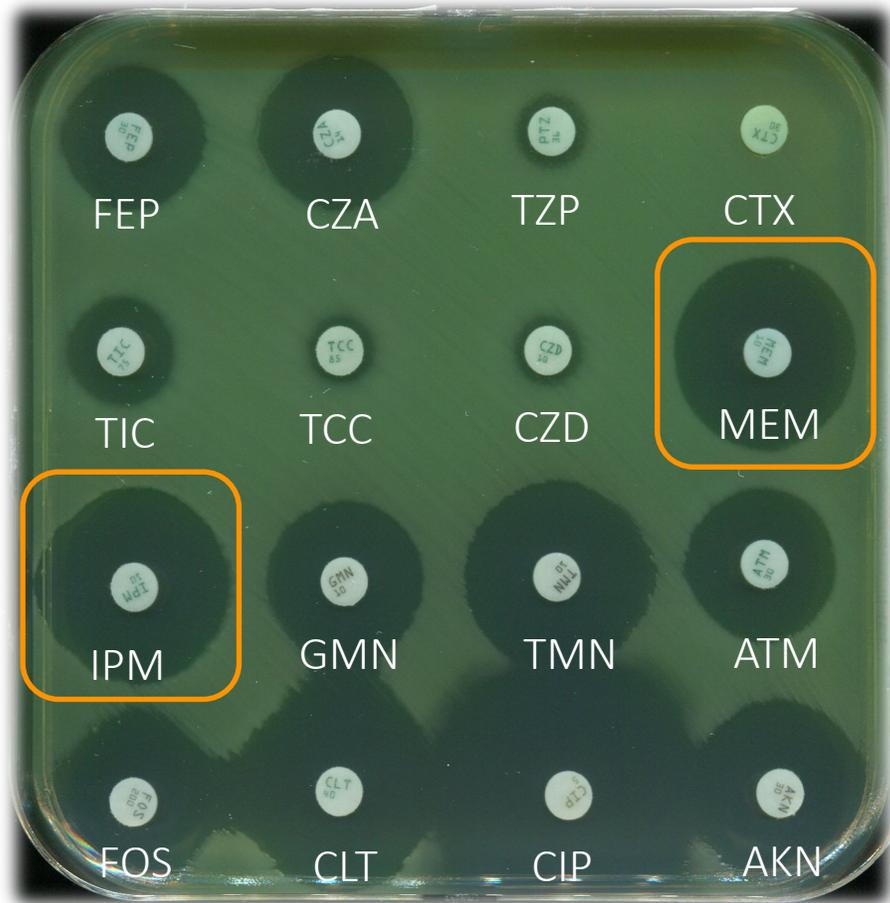
---



- ✦ Inhibition zone diameter of ceftazidime is lower than that of ceftazidime/avibactam and ceftolozane/tazobactam
- ✦ Due to the inhibitory activity of avibactam and ceftolozane on AmpC enzyme

# Overproduction of AmpC, PDC cephalosporinase

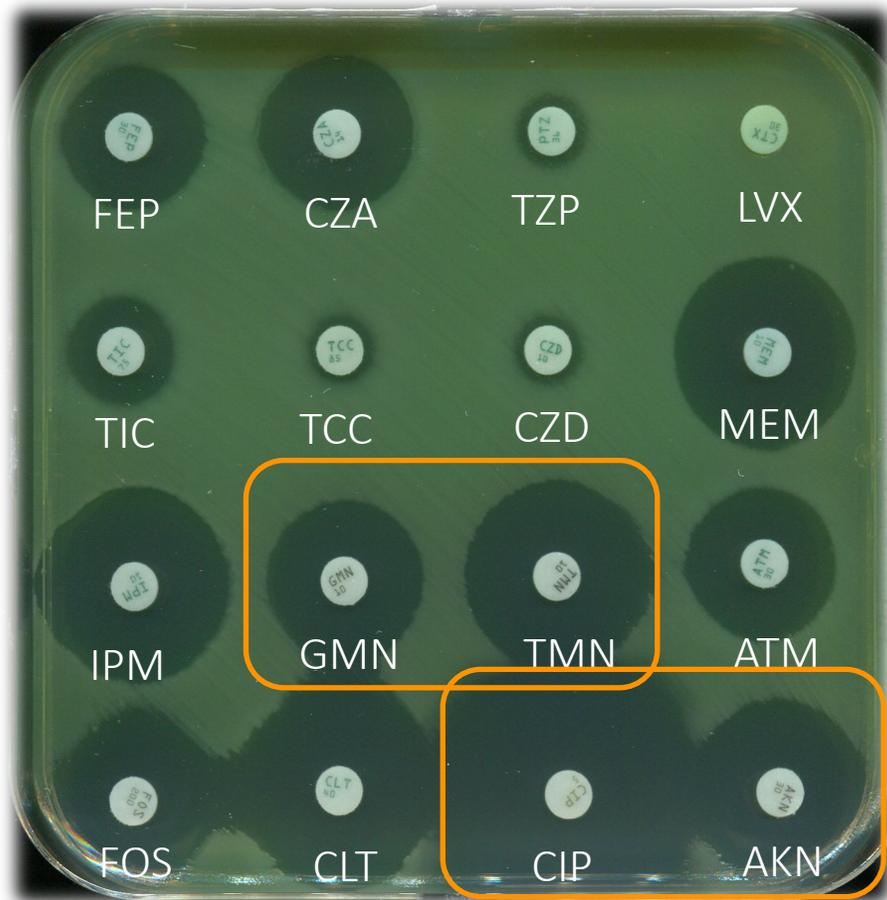
---



- ✦ No impact on carbapenems
- ✦ Due to the lack of hydrolytic activity of the AmpC enzyme on the carbapenems

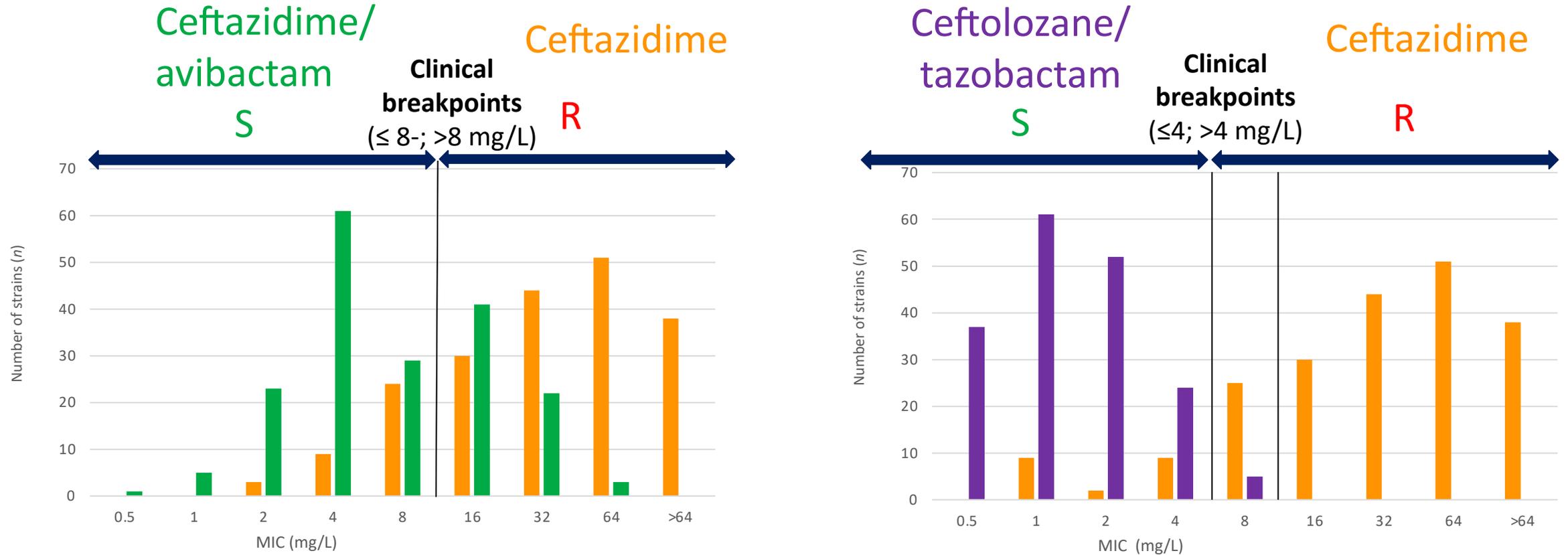
# Overproduction of AmpC, PDC cephalosporinase

---



- ✦ Absence of aminoglycosides- and/or fluoroquinolones-associated resistance

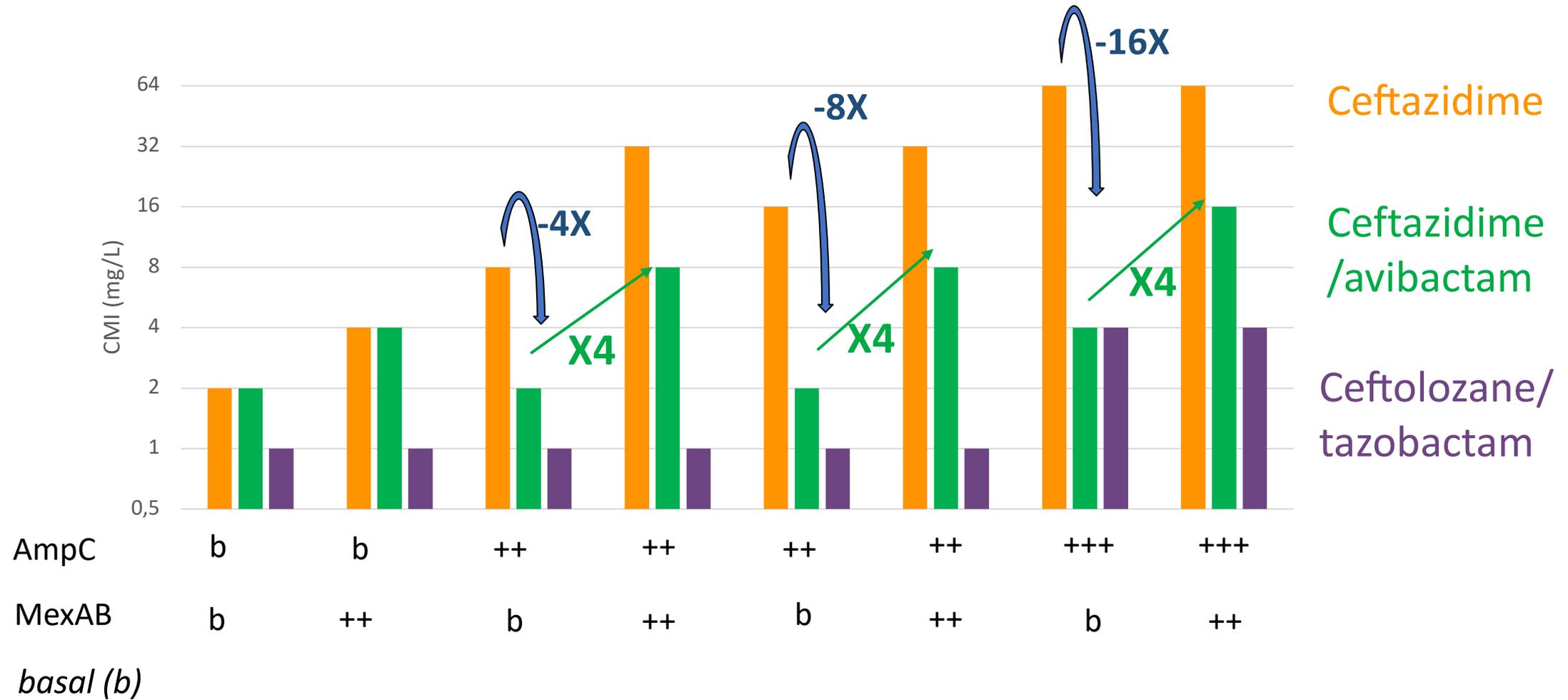
# Activity of ceftazidime/avibactam and ceftolozane/tazobactam



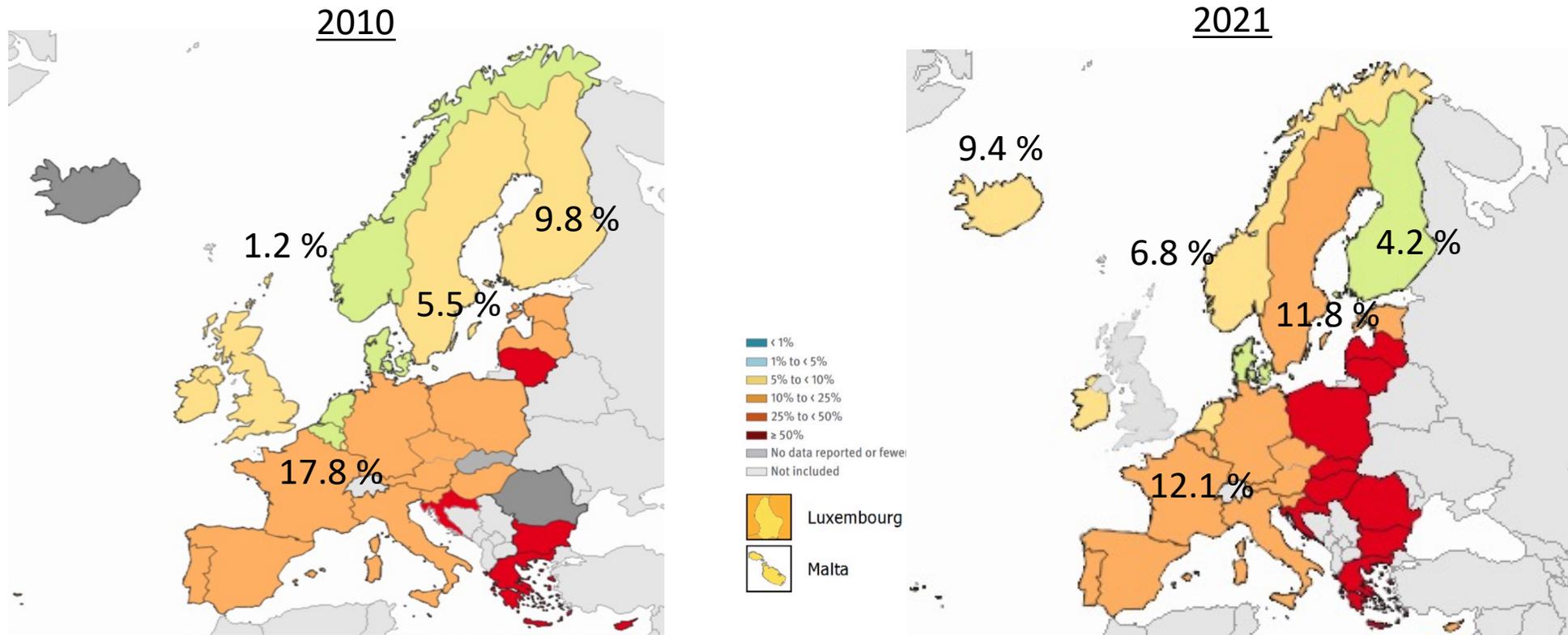
(n=411 strains)

AmpC<sup>+</sup> clinical isolates

# Avibactam is a substrate of MexAB-OprM efflux pump

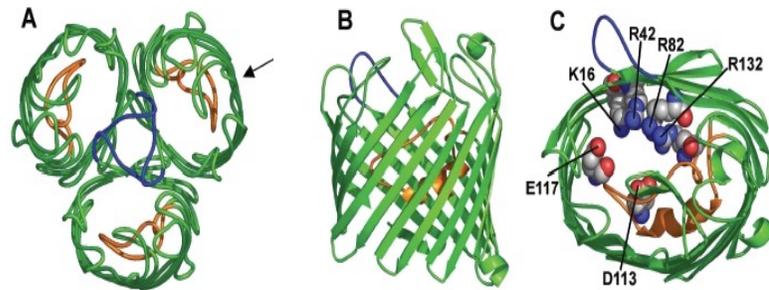
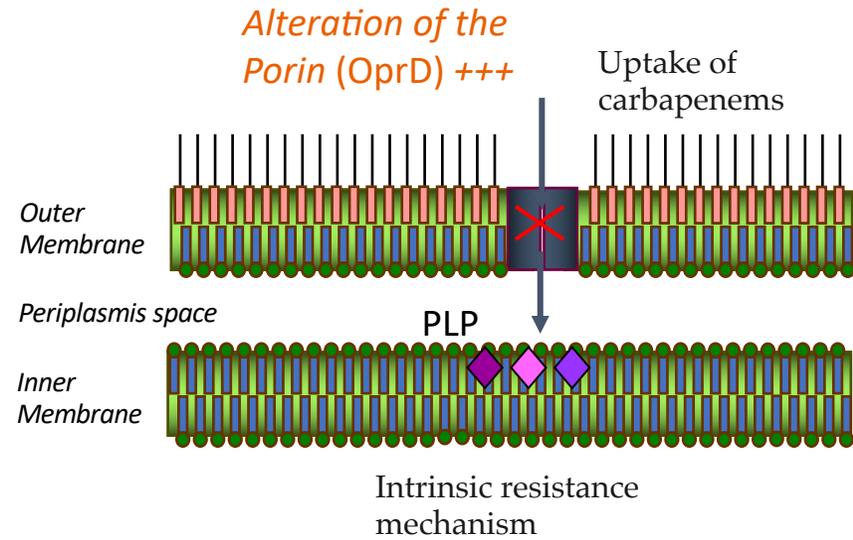


# Rates of carbapenem resistant in *P. aeruginosa*



Source: ECDC 2022

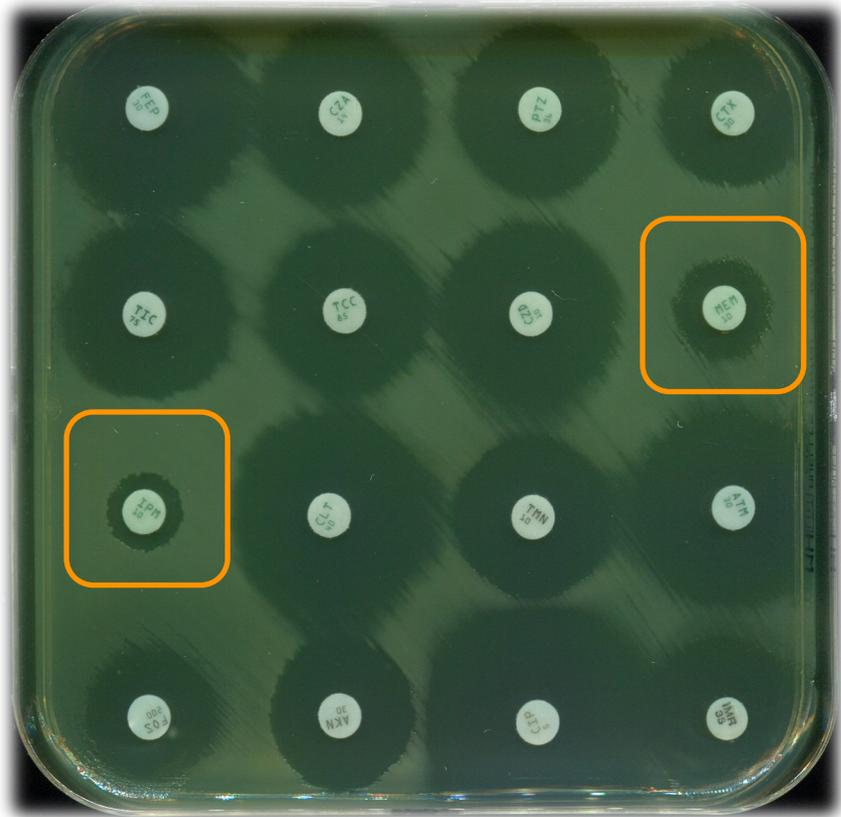
# Porin-dependent (OprD) resistance to carbapenems in *P. aeruginosa*



- ✓ Deficient mutants are common among clinical strains
- ✓ Alteration by mutations in gene *oprD* (rates  $10^{-6/7}$ )
- ✓ Downregulation by mutations in regulators of efflux systems MexEF-OprN and CzcBCA
- ✓ IPM<sup>r</sup>, MEM<sup>I-r</sup> phenotypes (X4-16-fold MIC)
- ✓ The loss of OprD potentiates the activity of pump MexAB-OprM (MEM<sup>r</sup>) and carbapenemases

# How to detect OprD mutant ?

---

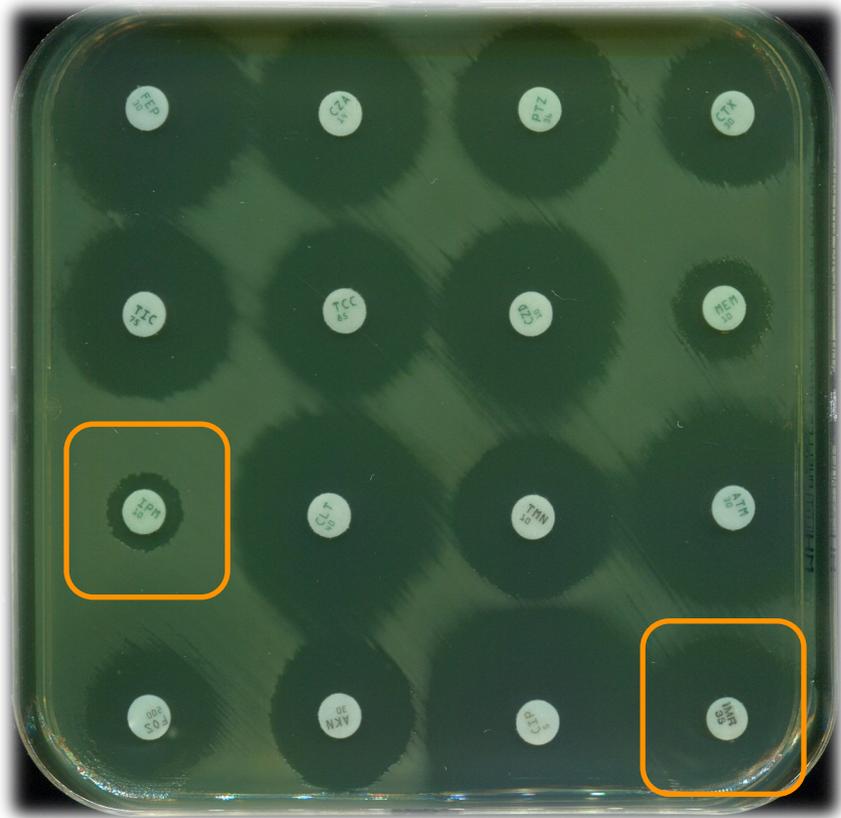


✓ Isolated resistance to cabapenems

FEP (cefepime), CZA (ceftazidime/avibactam), TZP (piperacillin-tazobactam), CTX (cefotaxim), TIC (ticarcillin), TCC (ticarcillin-clavulanate), CZD (ceftazidime), MEM (meropenem), IPM (imipenem), CLT (ceftolozane/tazobactam), TMN (tobramycin), ATM (aztreonam), FOS (fosfomycin), AKN (amikacin), CIP (ciprofloxacin), IPR(imipenem/relebactam)

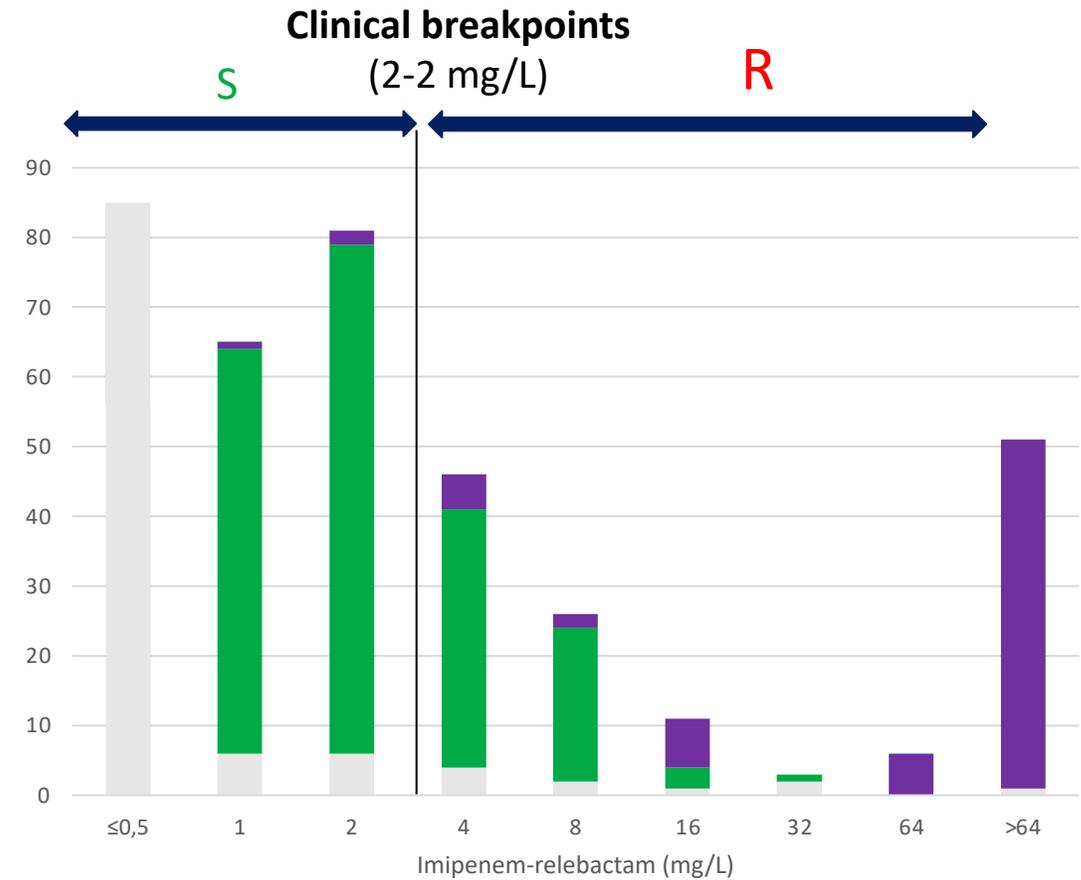
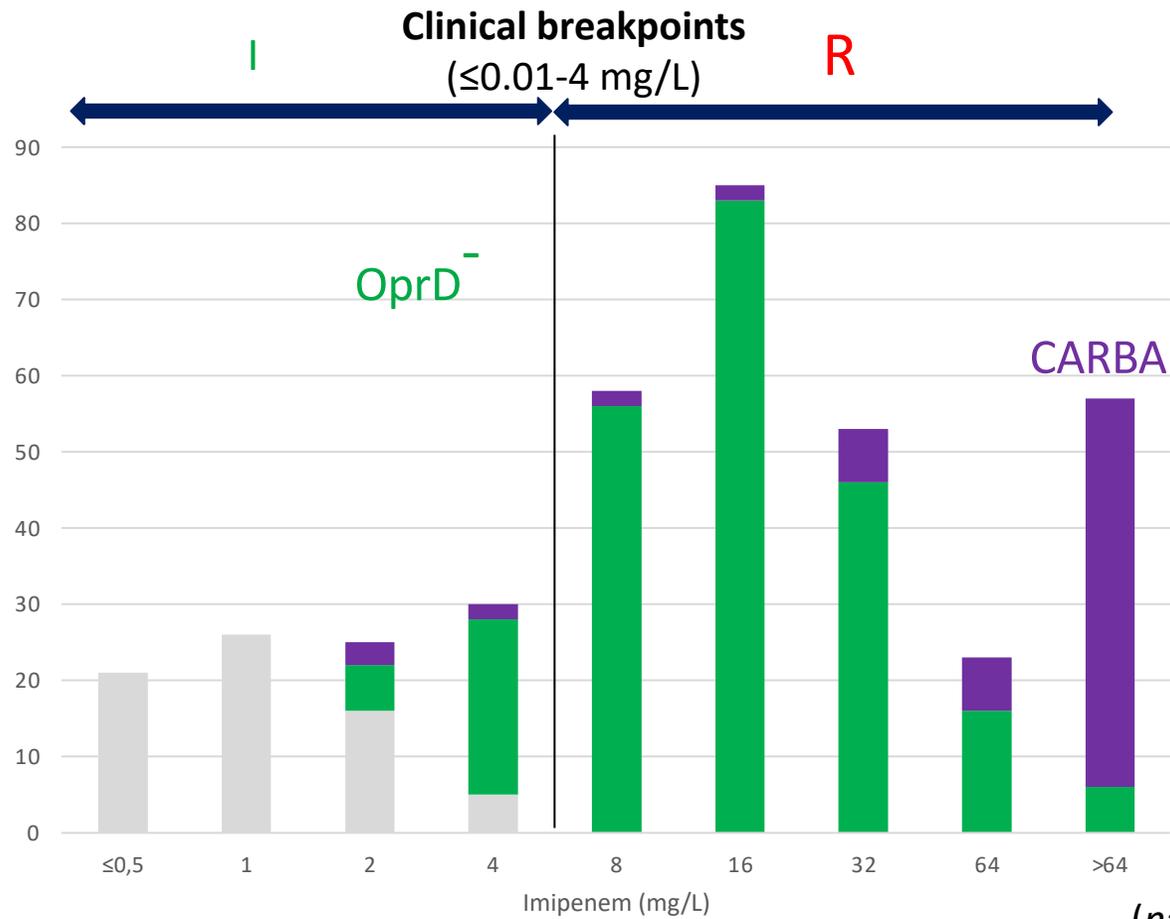
# Alteration of the OprD porin (OccD1)

---



- ✓ Isolated resistance to cabapenems  
or
- ✓ Restoration of imipenem activity with relebactam

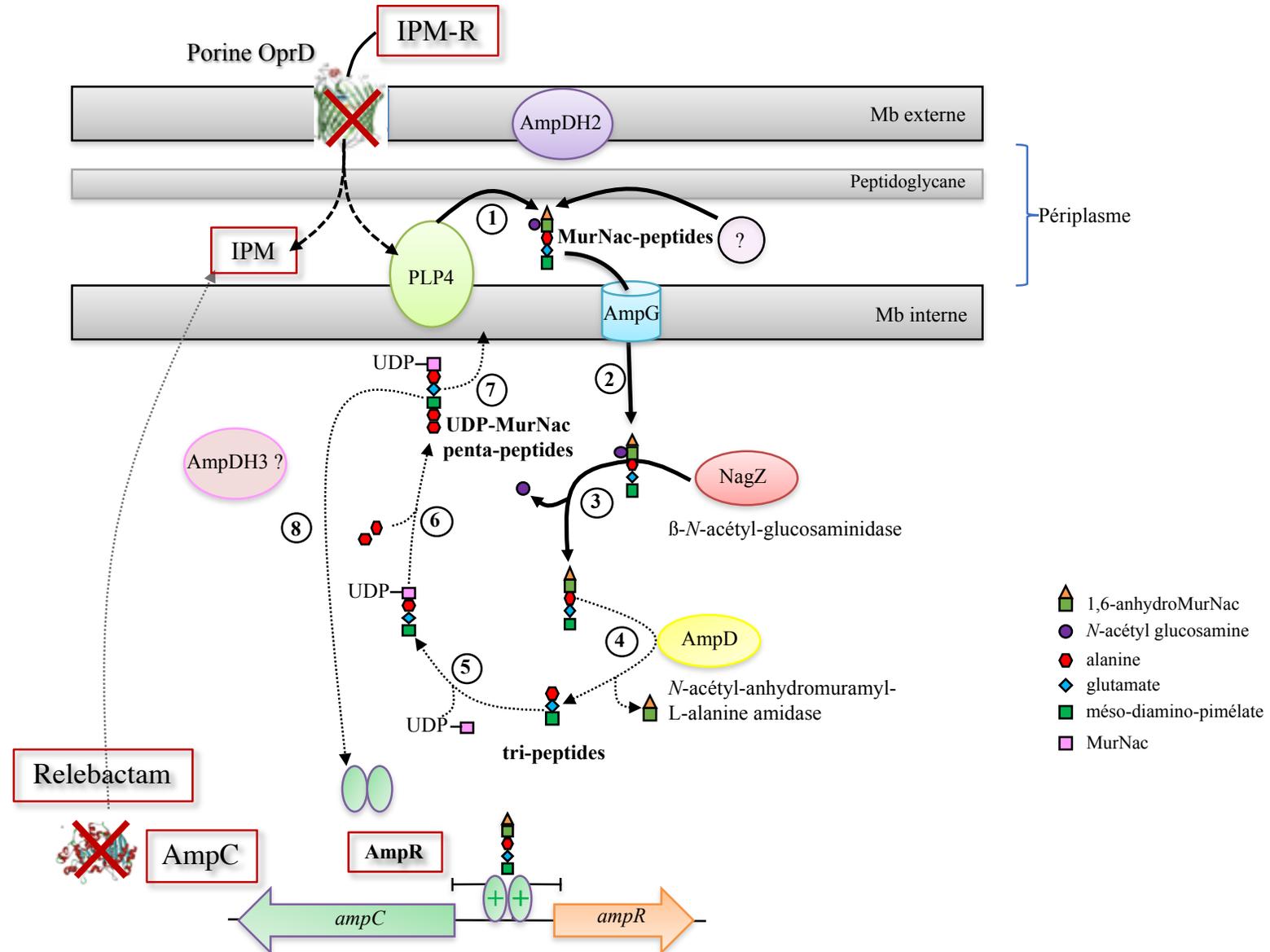
# Restoration of imipenem activity with relebactam *in vitro*



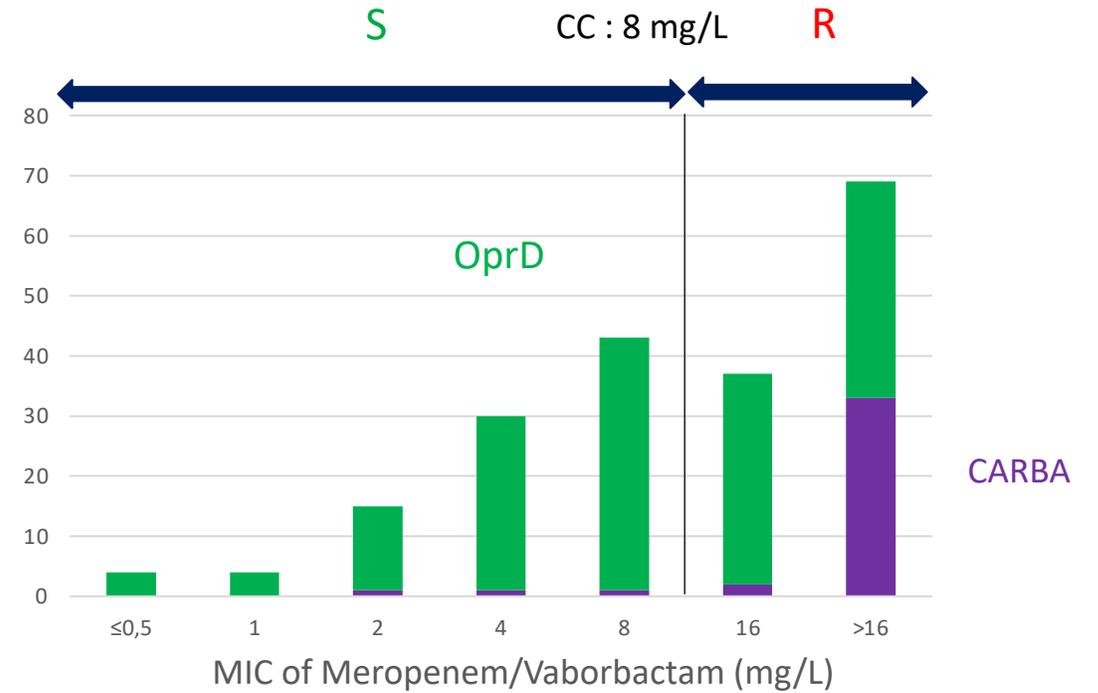
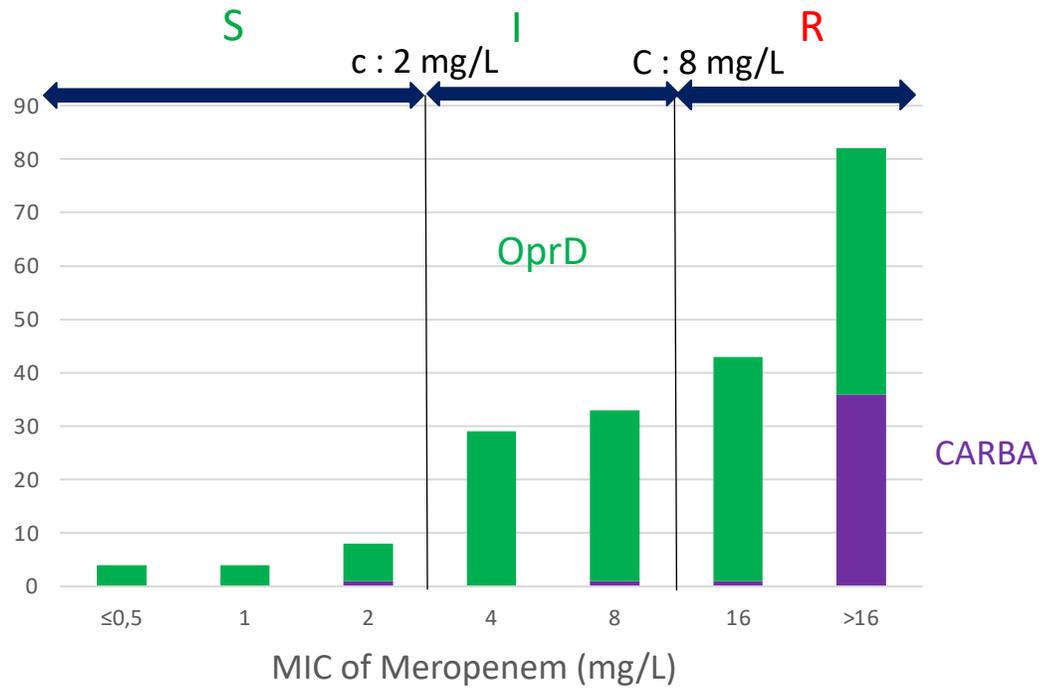
OprD<sup>-</sup>: OprD mutant

CARBA : production of a carbapenemase

# How Imipenem/relebactam works on OprD mutants ?



# Meropenem/vaborbactam activity *in vitro* in *P. aeruginosa*



(n=263 isolates)

# Interpretation of $\beta$ -lactamase inhibitors in OprD mutants

Antibiotics		S	R		Interpretation EUCAST 2023
		(mg/L)			
	OprD negative mutant				
Imipenem	8	0.001	4		<b>R</b>
Imipenem/relebactam	1	2	2		<b>S</b>
<b>Meropenem</b>	<b>4</b>	<b>2</b>	<b>8</b>		<b>I</b> (2g x3)
<b>Meropenem/vaborbactam</b>	<b>4</b>	<b>8</b>	<b>8</b>		<b>S</b> (2g +2g X3)

*I: susceptible increased exposure*

- \* Risk of inappropriate use of  $\beta$ -lactamase inhibitors
- \* **Vaborbactam** is not relevant in this species

# Carbapenemases identified in *P. aeruginosa*

1980's



Class A  
Penicillinases

- \* TEM-
- \* LCR-
- \* SHV-
- \* PSE- ou CARB-

1990's



**ESBL**

- \* TEM-
- \* SHV-2a, -12
- \* CTX-M-
- \* PER-
- \* VEB-
- \* GES-
- \* BES-
- \* PME-

Class B

**Carbapenemases**

- \* IMP-
- \* VIM-
- \* NDM-
- \* DIM-
- \* SPM-
- \* AIM-
- \* GIM-
- \* FIM-
- \* SIM

Class D: oxacillinases

**Narrow Spectrum**

- \* OXA-1, -2, -3, -4, -10...

**ESBL : ES-OXA**

- \* OXA-1 group : -31
- \* OXA-2 group : -15, -34, -36, 141, -161, 210
- \* OXA-10 group : -11, -14, -16, -17, -19, 28, 35,...

2000's



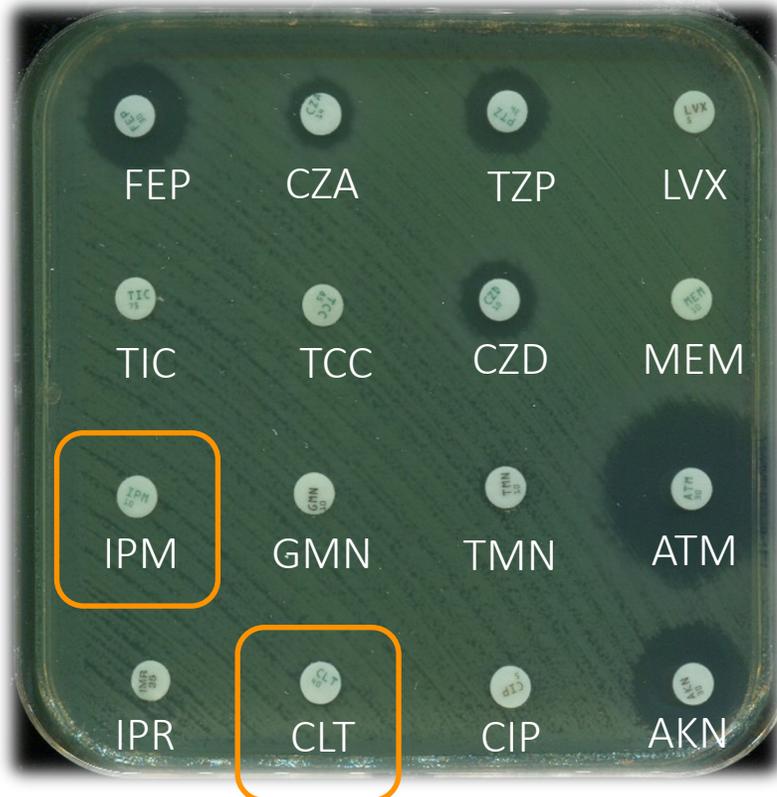
**Carbapenemases**

- \* KPC-2
- \* GES-4, -5, -6, -14, -18

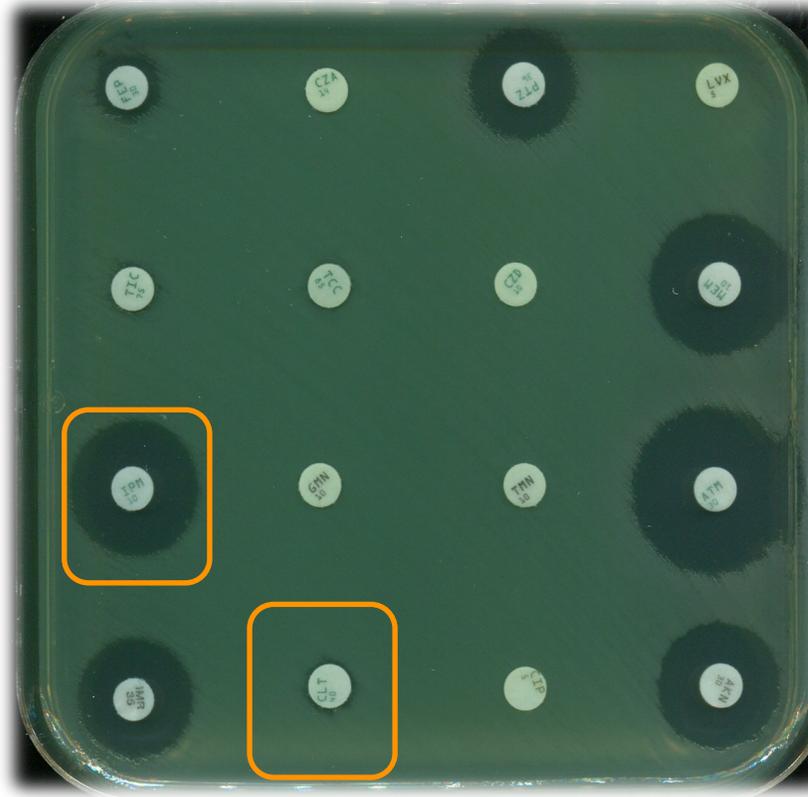
**Carbapenemases**

- \* OXA-198
- \* OXA-variants

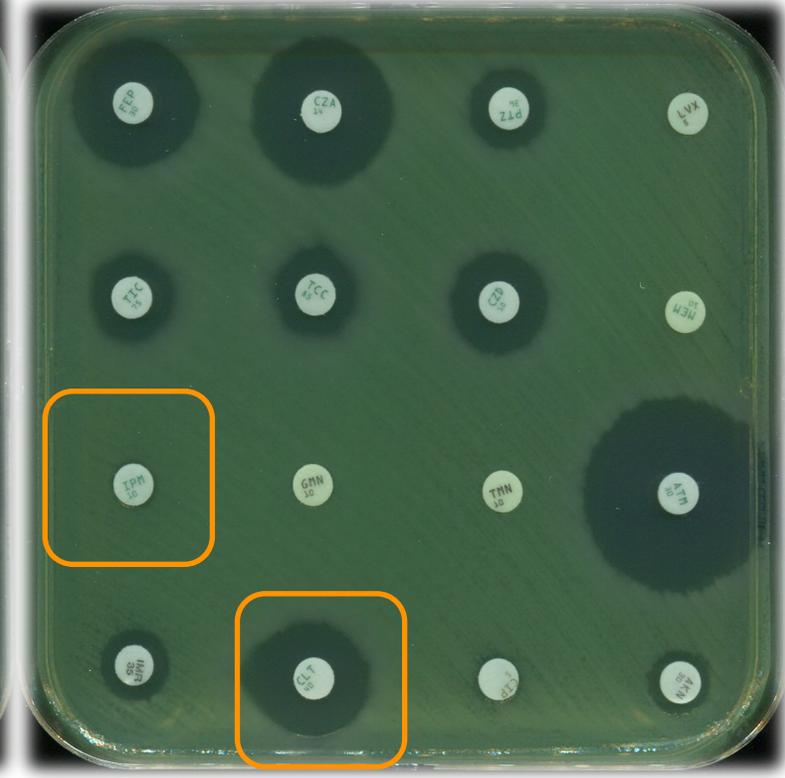
# Carbapenemase-producing clinical isolates



VIM-2

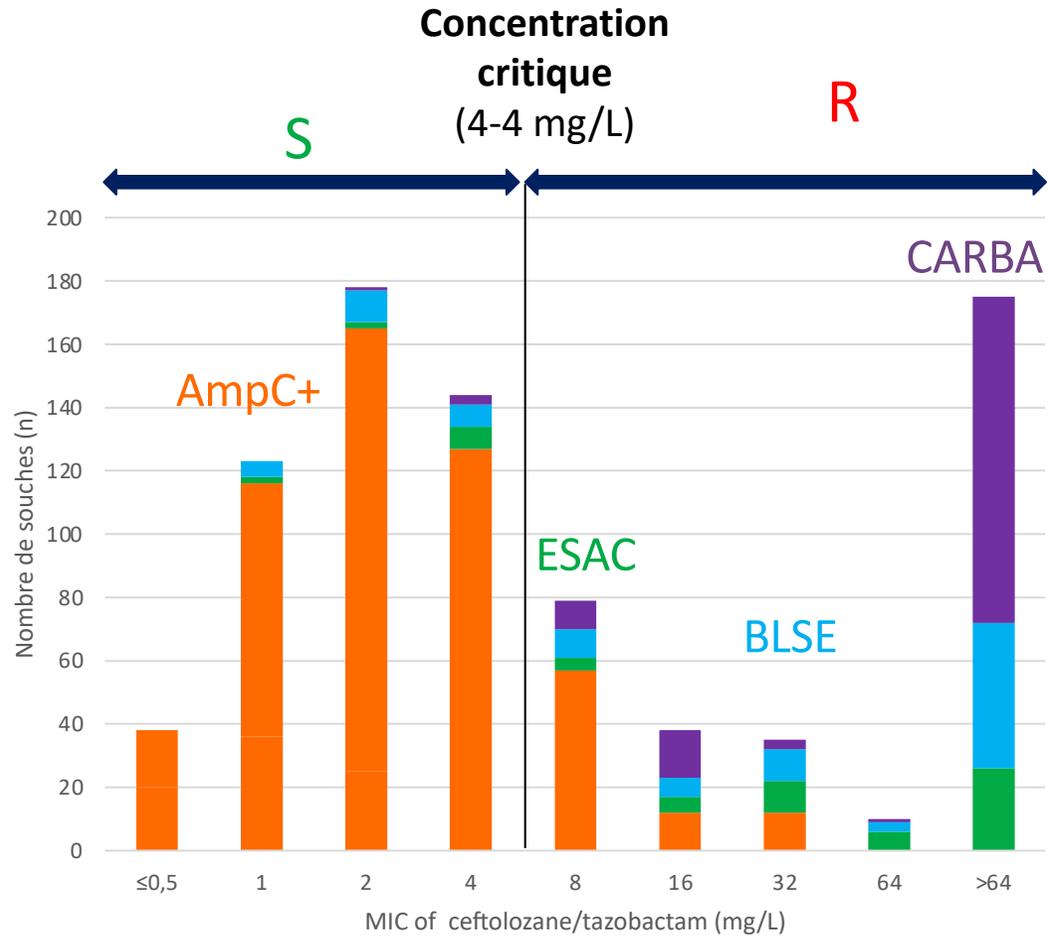


IMP-13

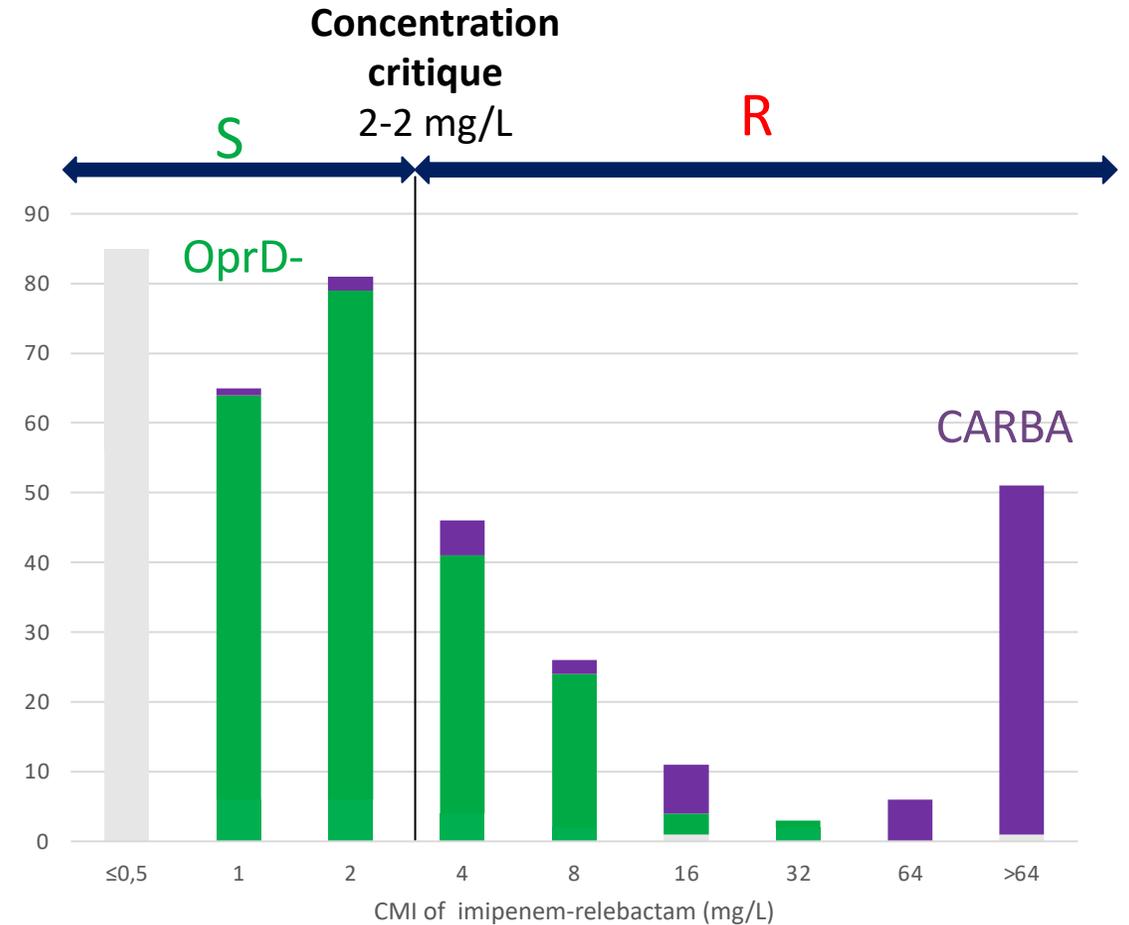


GES-5/OprD

# How to detect carbapenemases by disc diffusion method ?

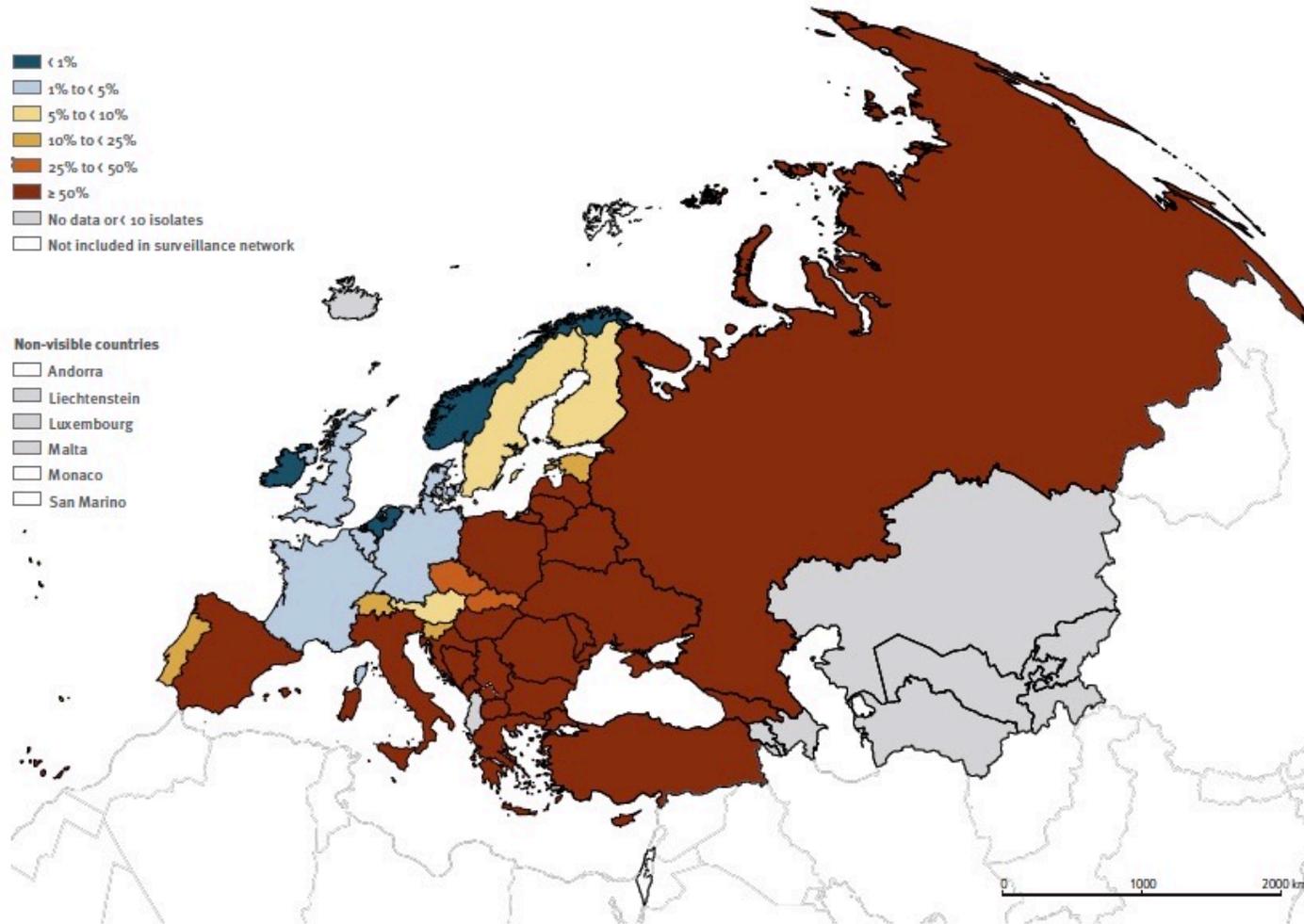


(n= 805 isolates)



(n=378 isolates)

# Rates of carbapenem resistance in *Acinetobacter* spp

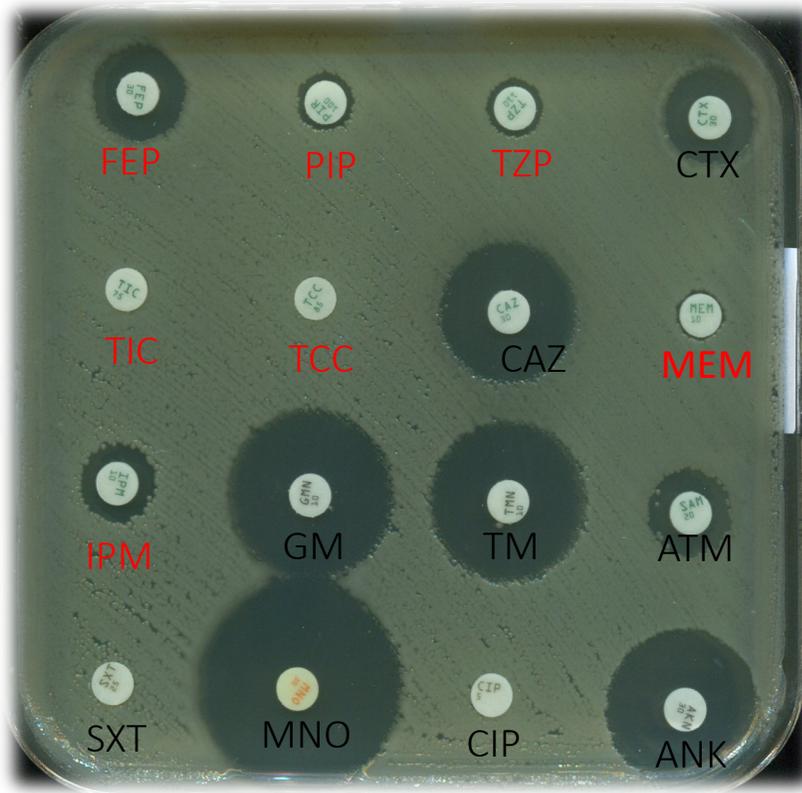


Antimicrobial Surveillance in Europe 2022, data 2020

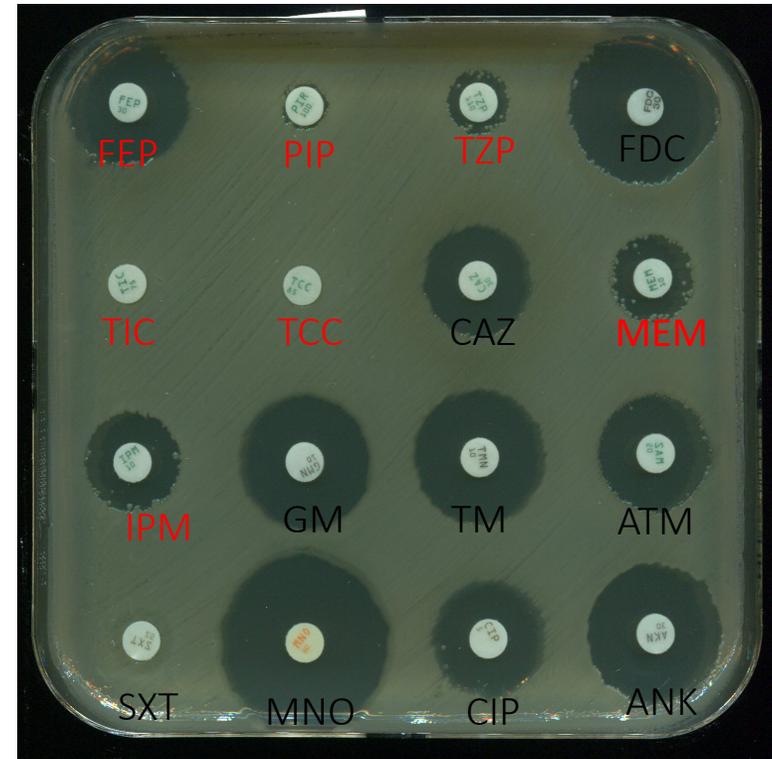
# Acquired $\beta$ -lactamases in *Acinetobacter baumannii*

	Narrow spectrum	ESBL	Carbapenemase
<b>Class A</b>	CARB-5 (RTG-2), <b>TEM-1,-2</b> , SCO-1 SHV-	<b>PER-1, -2, -3, -7</b> VEB-1, -3, -7 TEM -92,-116, -206 GES-7, -11, -12, -22 RTG-4 <b>CTX-M-2, -15, -44, -115</b> SHV-5, -11, -12	KPC-2, -3, -4, -10 GES-14 <b>PER-7</b>
<b>Class B</b>			IMP-1, -2, -4, -5, -6, -8, -11, -14, -19, -37, -63 <b>NDM-1, -2, -5, -9</b> VIM-1, -2, -4, -6, -11 SIM-1
<b>Class D</b>	OXA-10, -20, -21		<b>OXA-23</b> <b>OXA-24/40, 72</b> <b>OXA-58</b> OXA-143

# OXA-23 and OXA-58 producing strains of *A. baumannii*



MH

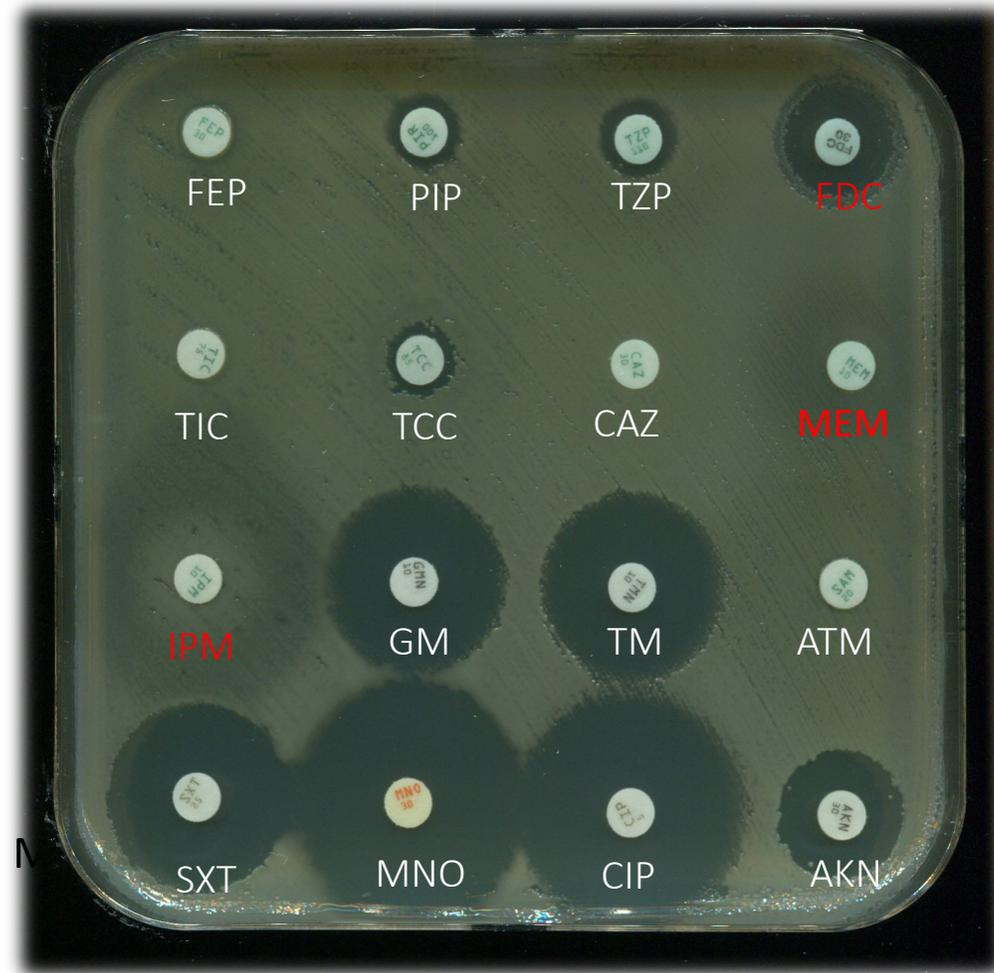


MH

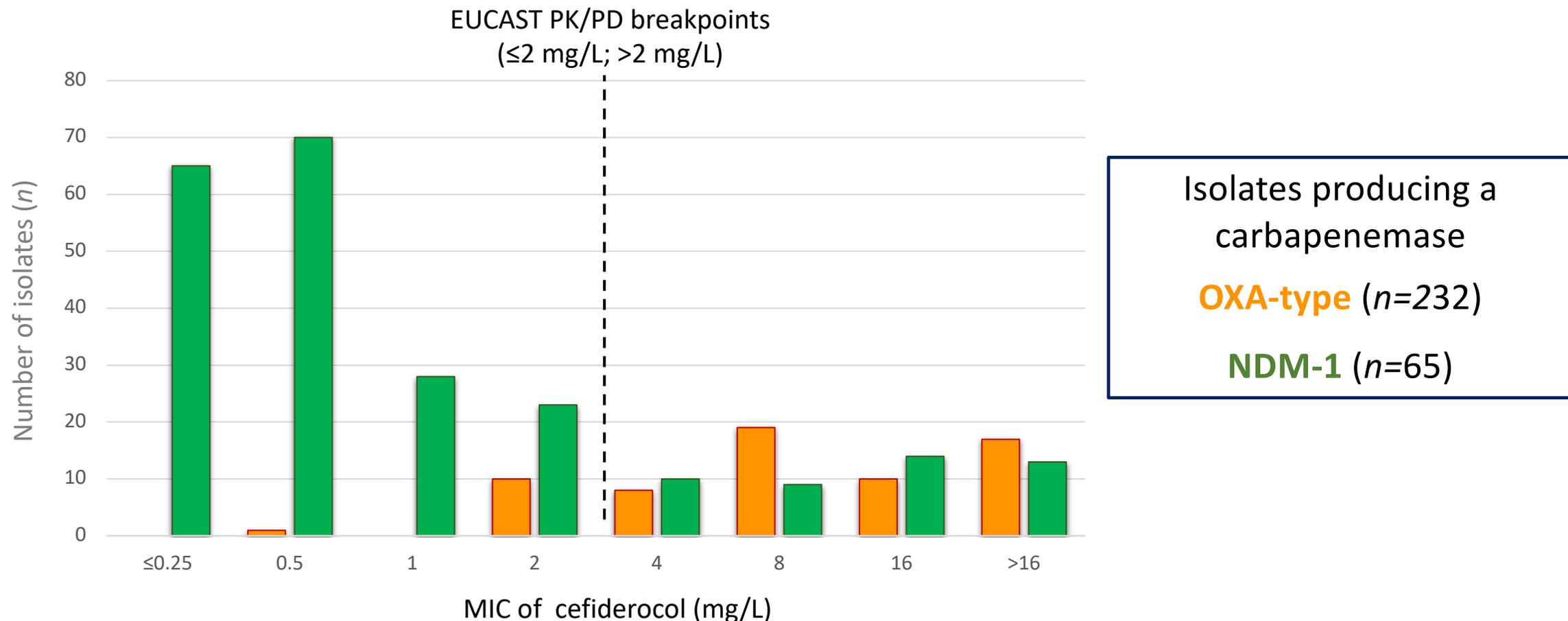
- ✦ High-level of resistance to ticarcillin and ticarcillin/clavulanic acid
- ✦ Resistance to carbapenems from 4 to 256 mg/L
- ✦ Lack of hydrolysis of 3<sup>rd</sup> cephalosporins

# NDM-1 producing strain *Acinetobacter baumannii*

---



# Susceptibility of CR-*A. baumannii* isolates to cefiderocol



- ✦ Excellent *in vitro* activity of cefiderocol in oxacillinase-producing isolates
- ✦ Impact of NDM-1 enzyme on cefiderocol susceptibility

# Activity of $\beta$ -lactams

Antibiotics	Intrinsic mechanisms				Foreign DNA Acquired				
	Overproduction			Alteration of the OprD porin	ESBL		Carbapenemases		
	AmpC	MexAB-OprM	MexXY (OprM)		SHV-2 <sub>a</sub>	PER-1	VIM-2	GES-5	NDM-1
TZP	R	S	S	S	R	S	R	R	R
CAZ	R	S	S	S	S*	R	R	R	R
FEP	I/R	S	I/R	S	R	R	R	R	R
CZA	S*	S*	S	S	S*	R	R	R	R
C/T	S	S	S	S	S	R	R	R	R
IMP	S	S	S	R	S	S	R	R	R
IMP/REL	S	S	S	S	S	S	R	R	R
MER	S	I	S	I/R	S	S	R	R	R
FDC	S	S	S	S	S	S*	S	S*	S*
Rates	+++	++	++	+++	+/-	+/-	+/-	-/+	+/-

# Thank you for your attention

## CNR-RA Besançon

- Damien Fournier
- Anaïs Potron
- Xavier Vuillemin
- Maxime Bour
- Pauline Triponney
- Emma Gauthier
- Léa Souro



Menu ☰

**CNR**  
RÉSISTANCE AUX  
ANTIBIOTIQUES

🔍

**Bienvenue sur le site du Centre National de Référence  
de la Résistance aux Antibiotiques**

**DÉPOSER**  
une demande d'analyse

Envoyer votre souche ↓

**ACCÉDER**  
au serveur de résultats

Email

Mot de passe  

Mot de passe oublié ?

Les actualités du CNR

↓

