



**Karolinska
Institutet**

Resistance to novel Gram-negative agents

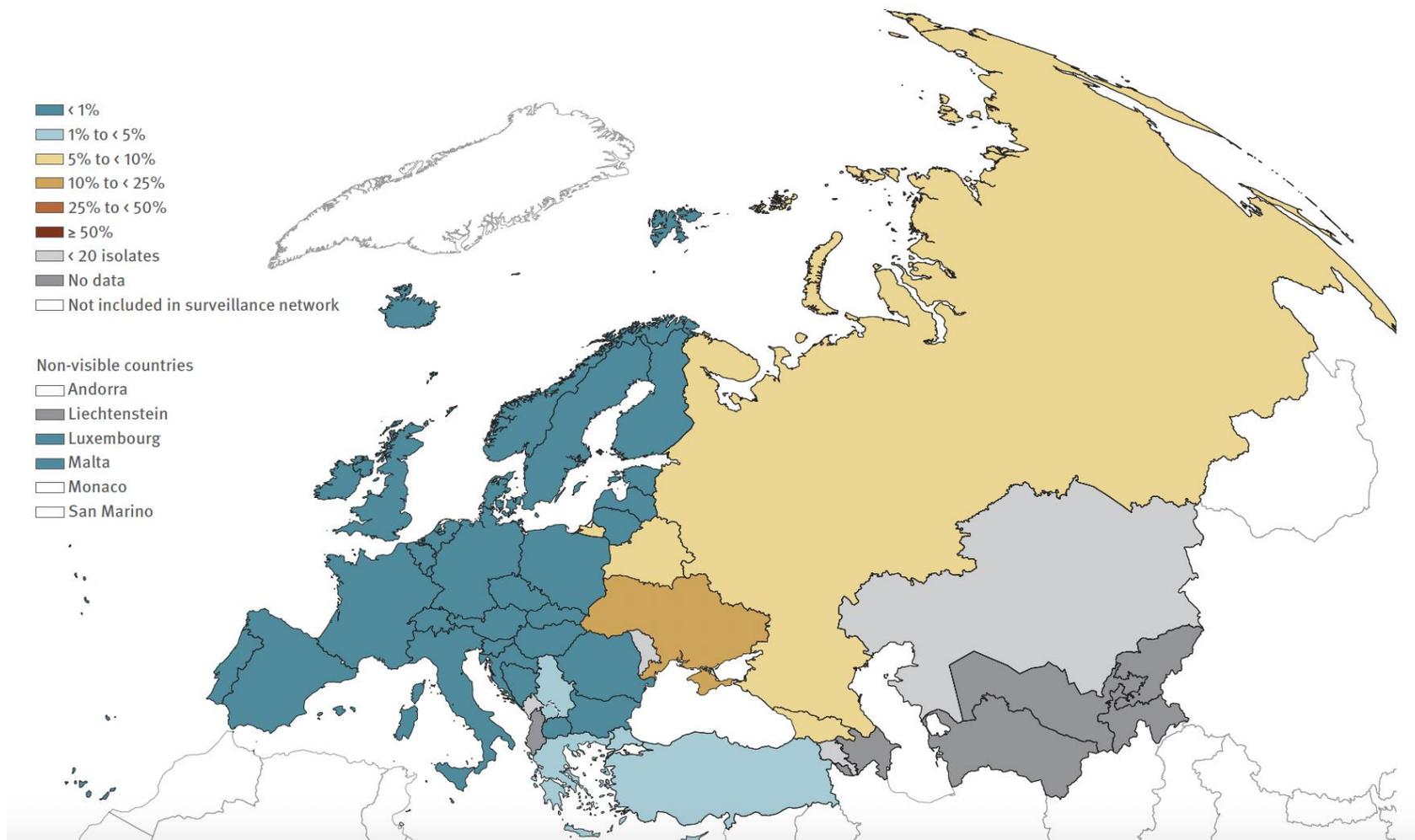
Christian G. Giske
Chair of EUCAST

Professor / Chief consultant physician
Karolinska Institutet, Stockholm, Sweden
NordicAST WS, Malmö, 10 May 2023

Current epidemiology

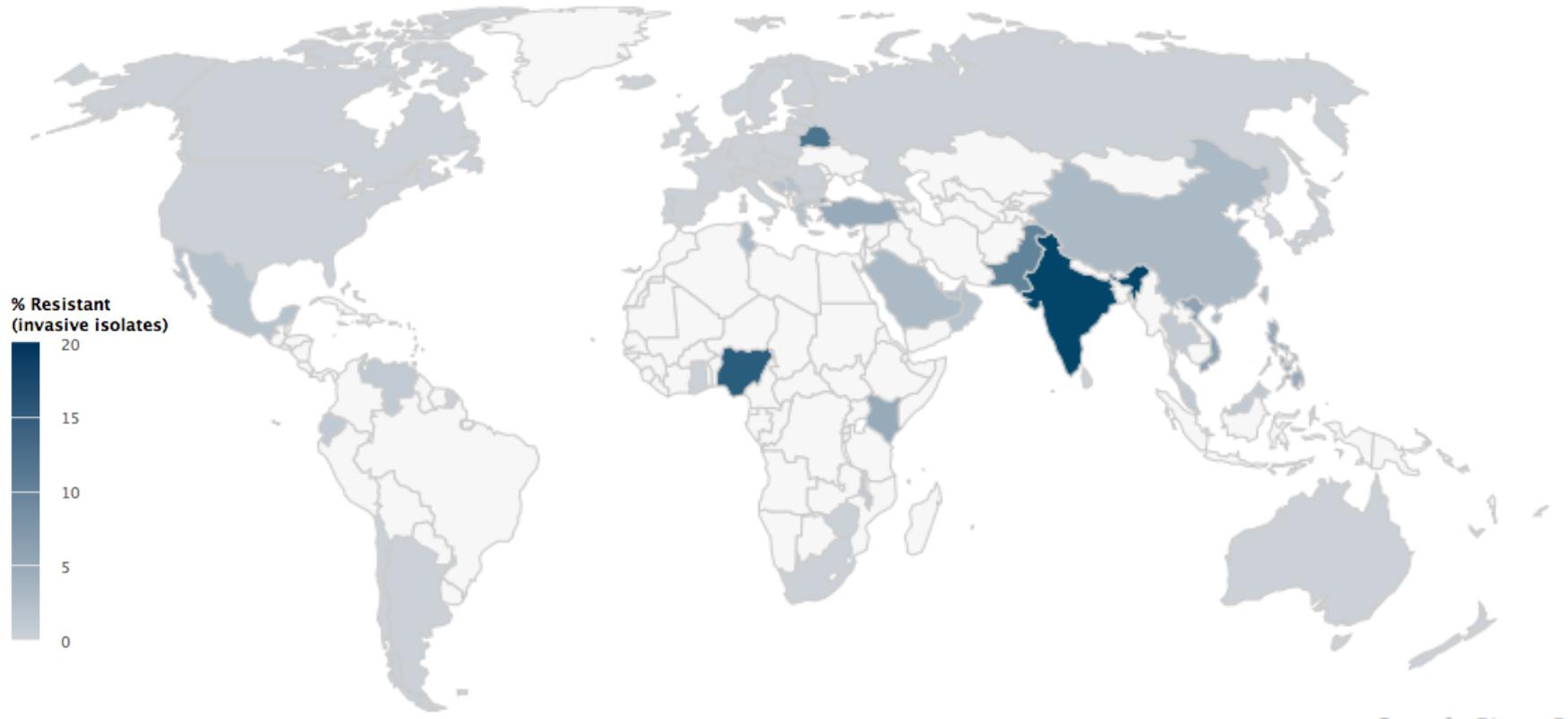


E. coli with carbapenem resistance 2021

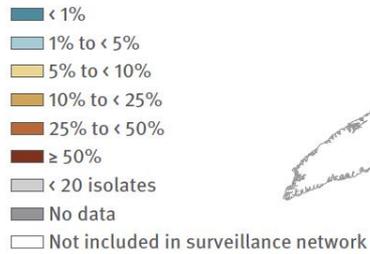


Invasive *E. coli*, aggregated data from EARS-Net and CAESAR

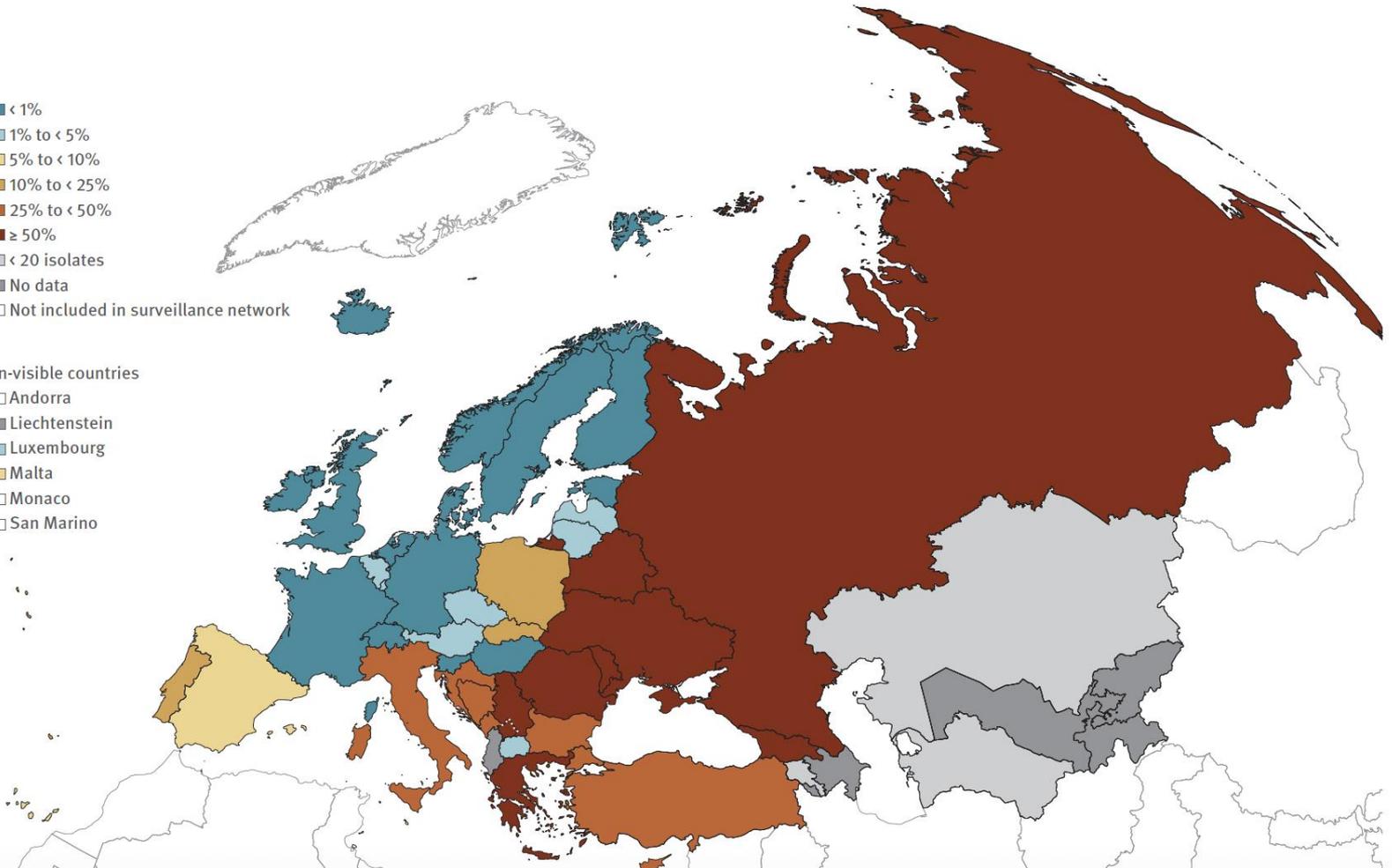
E. coli with carbapenem-R, global



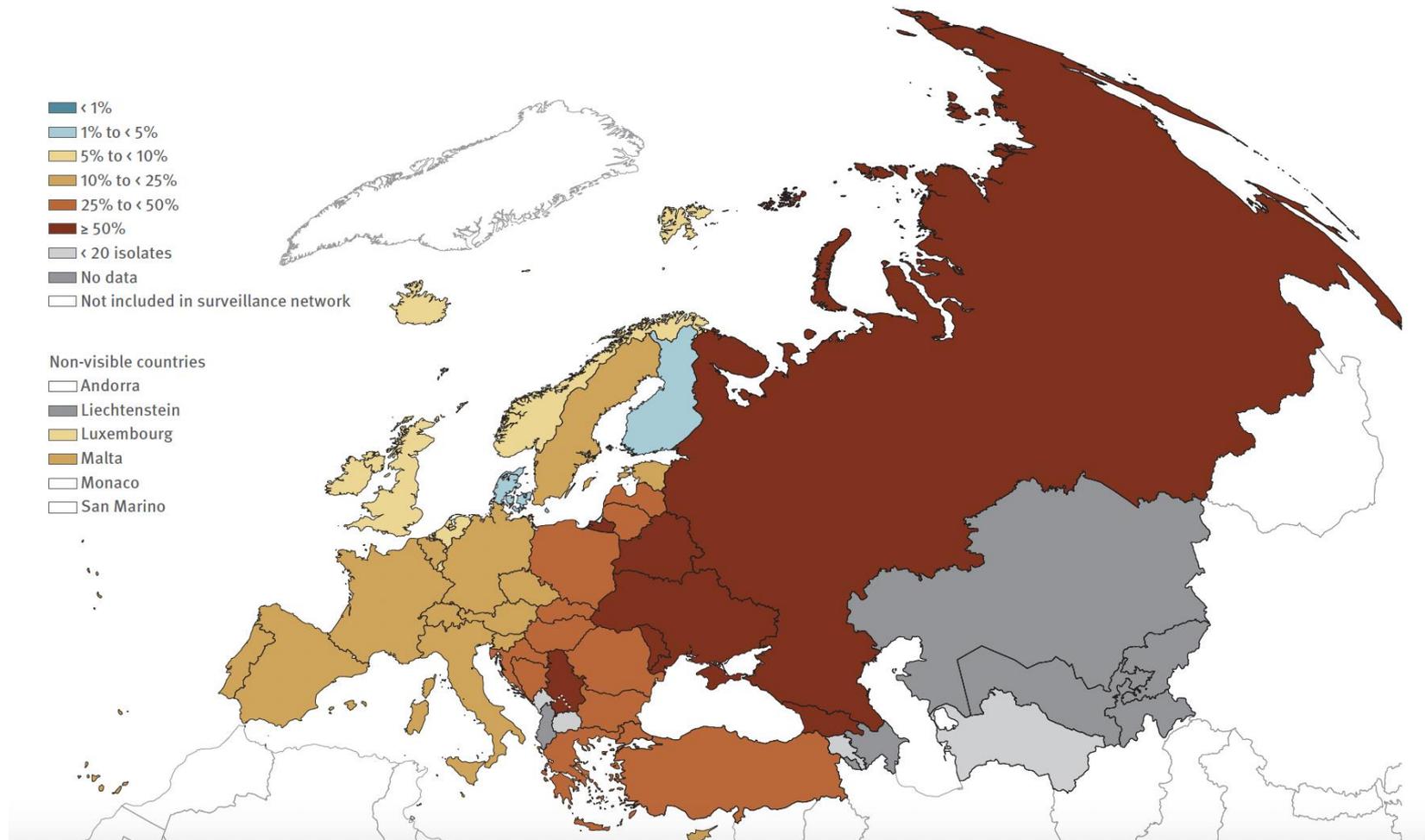
K. pneumoniae, 2021



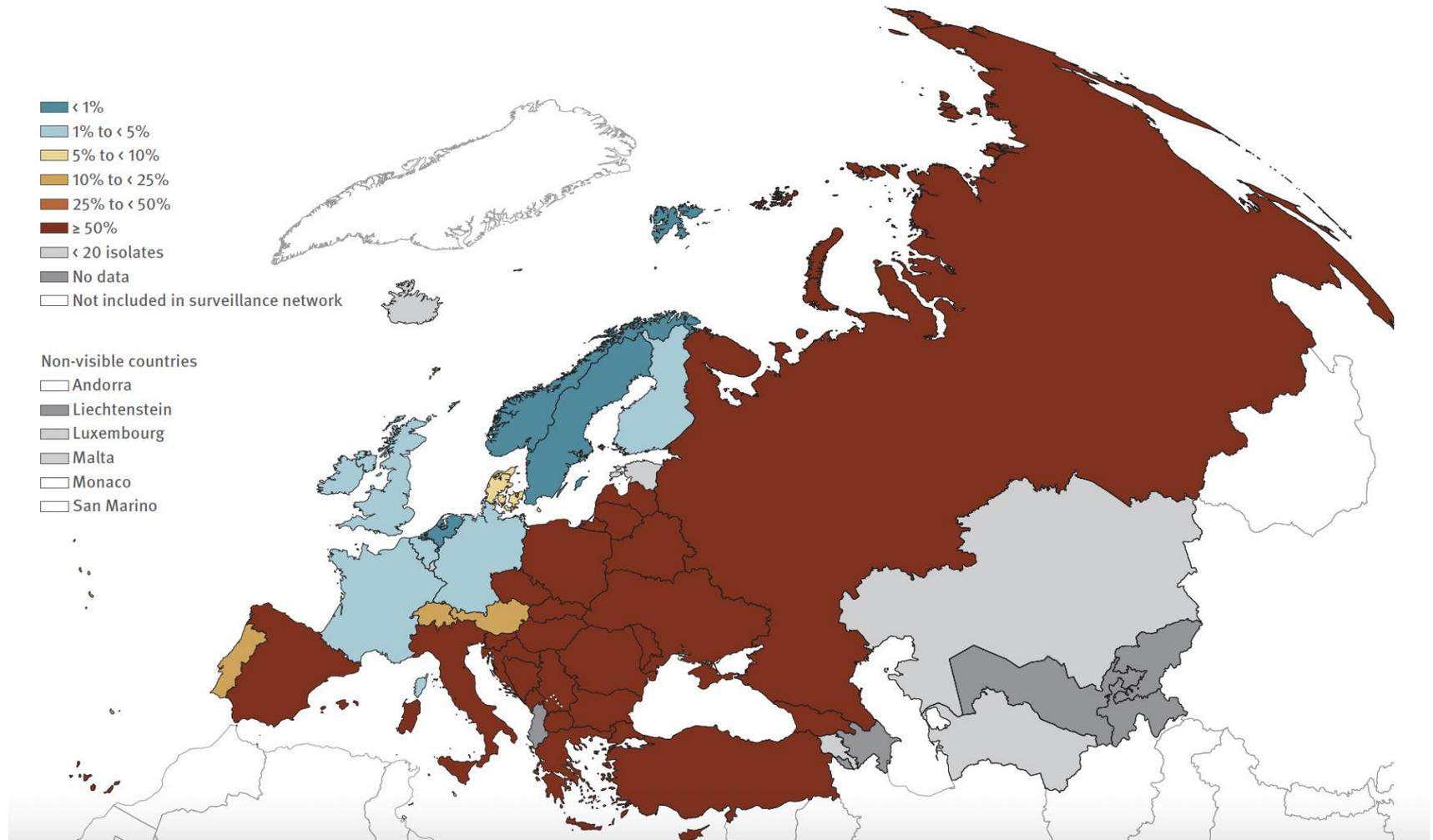
Non-visible countries



Carbapenem-R *P. aeruginosa*, 2021

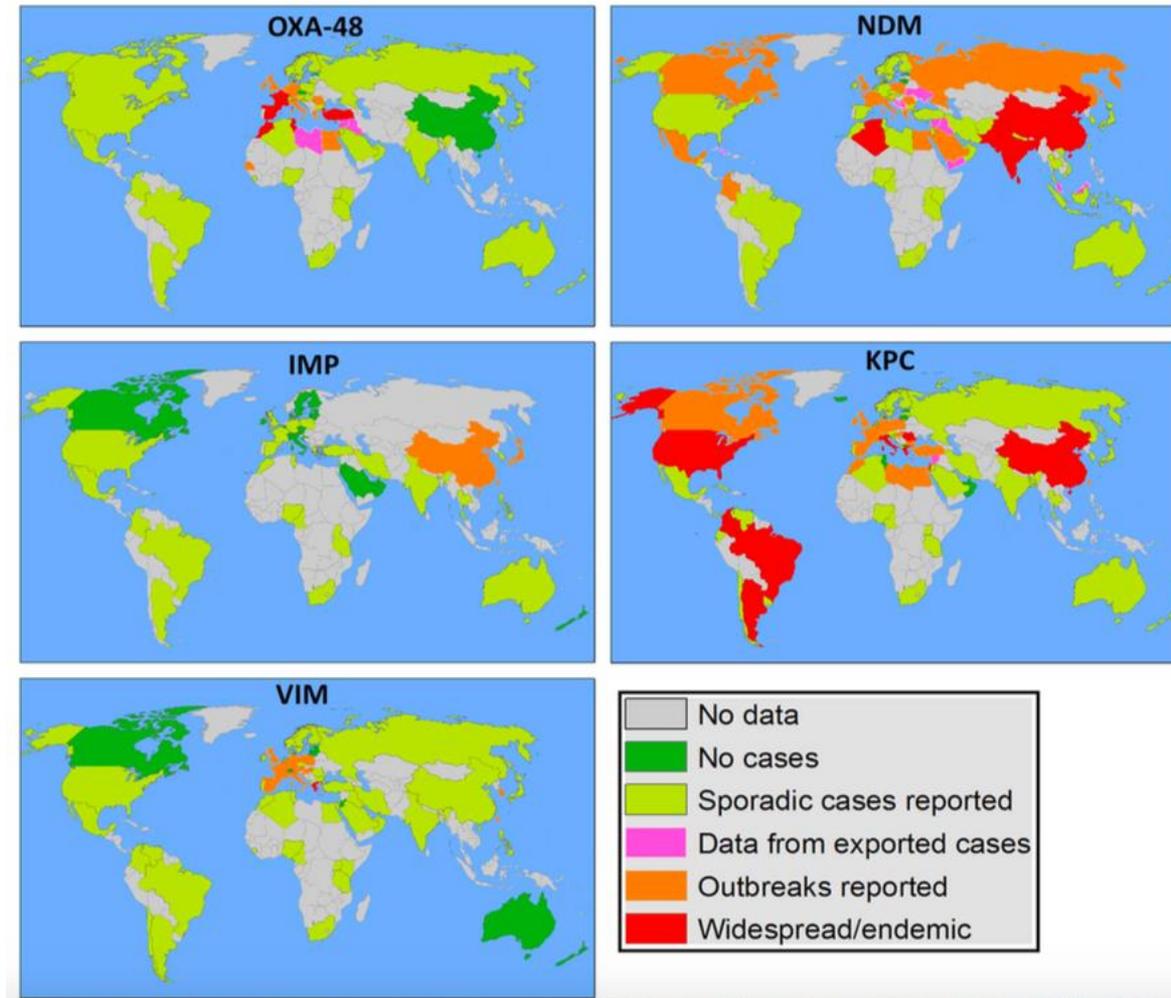


Carbapenem-R *Acinetobacter* 2021



Global dissemination

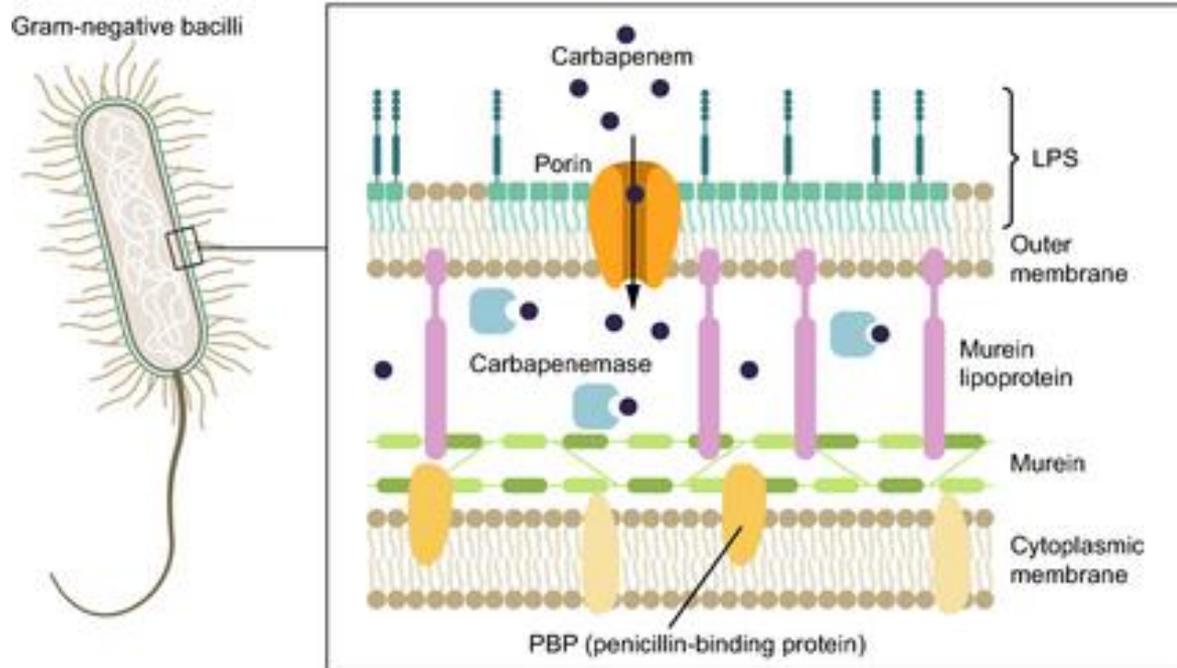
Trend: combination of carbapenemases is increasing (e.g. OXA-48+NDM or even NDM+KPC)



What can the drugs offer?



Multidrug-resistance: interplay of mechanisms



Beta-lactamases, porins
efflux pumps, PBP-
changes

When developing new
drugs all have to be
considered

Tängdén T and Giske CG. J Intern Med. 2015;277(5):501-12.

Enterobacterales – activity vs mechanisms of resistance

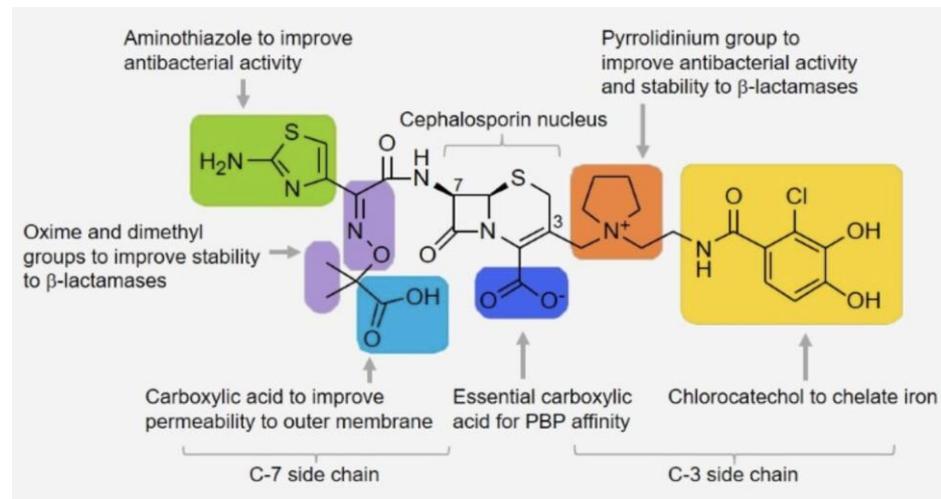
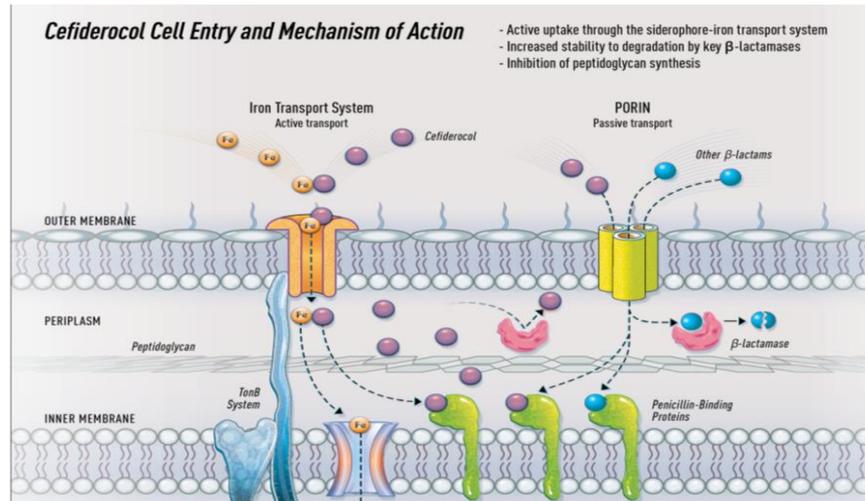
Antibiotics	ESBL	AmpC	KPC	MBL	OXA-48
Ceftolozane-tazobactam	+	-	-	-	-
Ceftazidime-avibactam	+	+	+	-	+
Meropenem-vaborbactam	+	+	+	-	(+/-)
Imipenem-relebactam	+	+	+	-	-
Cefiderocol	+	+	+	+	+

+ = possibly preferred agents

Is MER-VAB preferable to CAZ-AVI in KPC-infections?

- With both treatment options (and also likely with IMI-REL), emergence of resistance can occur
 - Mechanisms can both be related to porins and expression of enzymes, and cross resistance is to be expected
 - Development of resistance more common with ceftazidime-avibactam (Ackley R et al. AAC 2020; 64: e02313)
 - Data from Italy: meropenem/vaborbactam resistance emerged in 13% of KPC-Kp strains isolated from bloodstream infection (Gaibani M et al. Antibiotics 2021; 10: 536)
 - Possibly MER-VAB should be preferred, but resistance can emerge with both agents
 - Direct comparison with IMI-REL is lacking so far
-

Cefiderocol: why is it stable towards all enzymes?



Why should ceftazidime-avibactam be preferred against OXA-48-producers?

- Avibactam inhibits OXA-48
 - Ceftazidime is not affected by hydrolysis – rather resistance is conferred by other enzymes
 - MER-VAB can often be susceptible, but this is not related to VAB inhibition of OXA-48 – rather to OXA-48 susceptibility to high exposure of meropenem
 - Cefiderocol: could also be used, but no advantage over CAZ-AVI
 - Yahav D et al. Clin Microbiol Rev. 2020; 34: e00115
-

P. aeruginosa – activity vs mechanisms of resistance

Antibiotics	AmpC	Class A carba- penem ase	MBL (class B)	Porin loss
Ceftolozane- tazobactam	+	-	-	+
Ceftazidime- avibactam	+	+	-	+
Meropenem- vaborbactam	+	+	-	-
Imipenem-relebactam	+	+	-	+
Cefiderocol	+	+	+	+

+ = contrary to imipenem alone remains stable

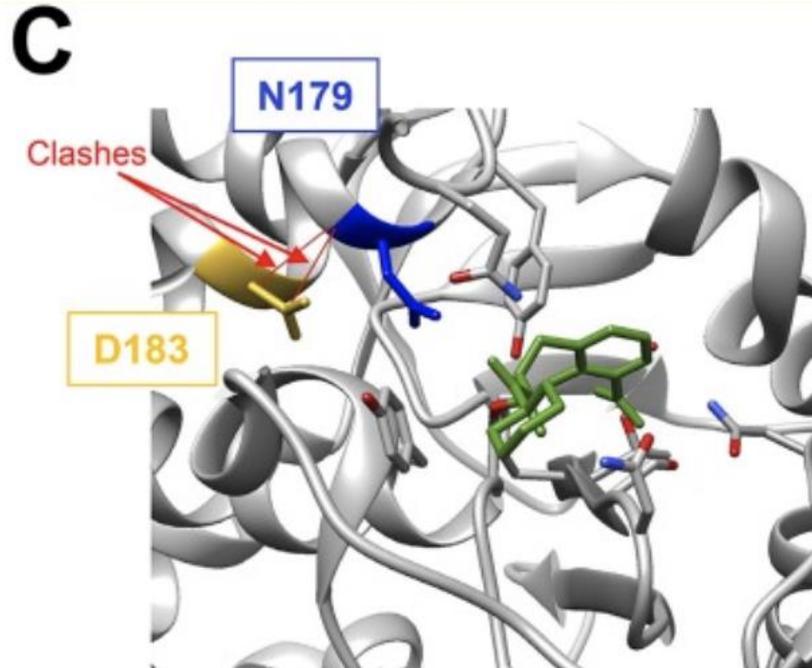
How does imipenem-relebactam compare to other alternatives?

PAO1 variant	MIC ($\mu\text{g/ml}$)			
	TOL-TZ ($S \leq 4$)	CAZ-AVI ($S \leq 8$)	IMP ($S \leq 4$)	IMI-REL ($S \leq 4$)
PAO1	≤ 0.5	1	0.5	0.125
PAOD1 (OprD ⁻)	≤ 0.5	1	8	0.25
PAOD1 Δ AmpC	≤ 0.5	1	0.5	0.5
PAOD1 Δ AmpC + pUCP AmpC _{WT} (PDC-1)	1	2	4	0.5
PAOD1 Δ AmpC + pUCP AmpC _{T96I} (PDC-222)	32/4	8	0.5	0.5
PAOD1 Δ AmpC + pUCP AmpC _{E247K} (PDC-221)	32	16	0.5	0.5
PAOD1 Δ AmpC + pUCP AmpC _{ΔG299-E247K} (PDC-223)	>32	32	0.5	0.5
PAOD1 Δ AmpC + pUCP AmpC _{F147L} (PDC-316)	16	8	0.5	0.5
PAOD1 Δ AmpC + pUCP AmpC _{E247G} (PDC-80)	32	4	1	0.5

Very few chromosomal mechanisms affect *P. aeruginosa*

Relevant to test vs MDR-*P. aeruginosa*, including imipenem resistant strains

TOL-TAZ and CAZ-AVI: resistance through mutations in PDC



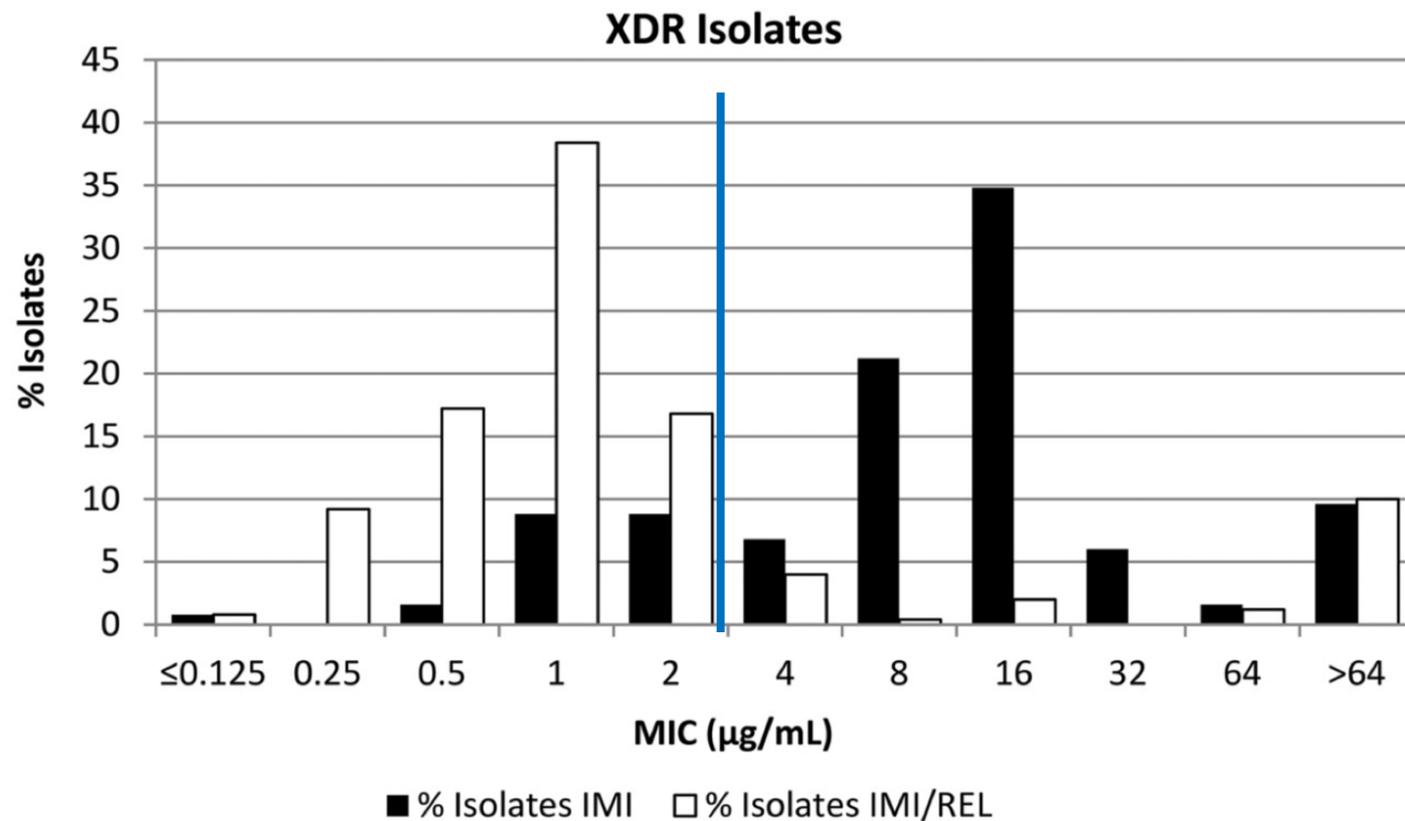
Single step mutation occurring in vivo in chromosomal AmpC (PDC) conferred resistance to ceftazidime-avibactam and ceftolozane-tazobactam

Associated with too low dose or off-label use?

Also shown in hollow-fiber models

Boulant et al. AAC 2019; 63:e01637

Imipenem-relebactam vs XDR- *P. aeruginosa*



Low-level resistance: PBP

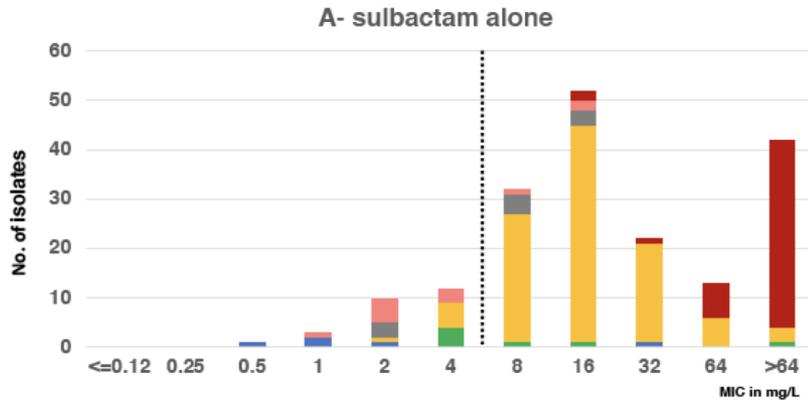
A. baumannii – activity vs mechanisms of resistance

Antibiotics	Class A carba-penemase	MBL (class B)	Class D carba-penemase
Ceftolozane-tazobactam	-	-	-
Ceftazidime-avibactam	-	-	-*
Meropenem-vaborbactam	+	-	-
Imipenem-relebactam	+	-	-
Cefiderocol	+	+	+

*Avibactam inhibits class D carbapenemases

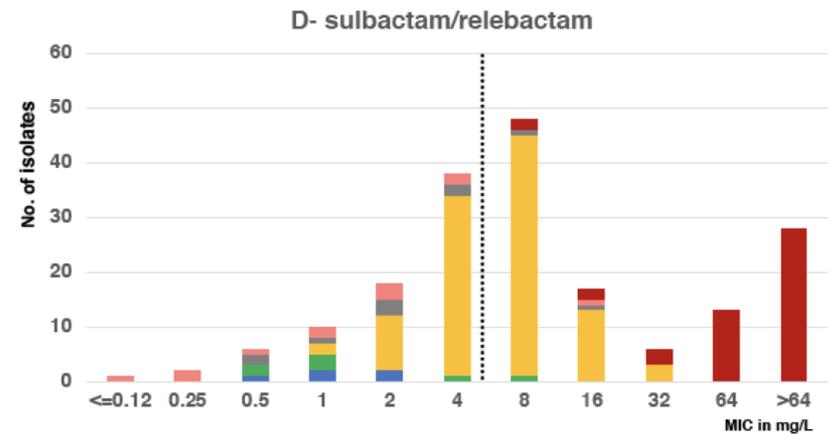
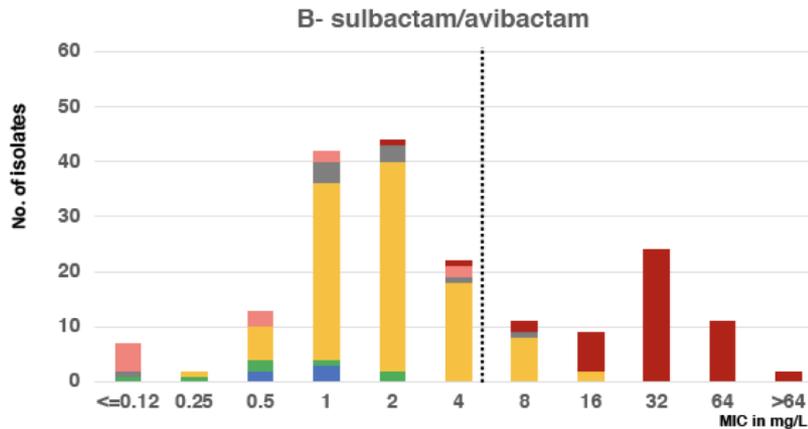
Ampicillin-sulbactam plus ceftazidime-avibactam theoretically feasible

Avibactam can inhibit CHDL in *Acinetobacter*



Main resistance mechanisms (no. of Isolates):

- Non-ESBL/Non-CRAB (4)
- ESBL (7)
- OXA-23 (105)
- Other OXAs (11)
- IMP-1 (12)
- NDM-1 (48)



Pasteran F et al. Antibiotics 2021; 10: 577

Indications and contraindications for testing



Testing of *Enterobacterales*

- Ceftolozane-tazobactam: refrain from testing
 - KPC/class A enzymes
 - Ceftazidime-avibactam, meropenem-vaborbactam and imipenem-relebactam can all be tested
 - Cefiderocol can be considered
 - OXA-48 group
 - Ceftazidime-avibactam can be tested
 - MER-VAB and IMI-REL offer nothing compared to MER and IMI alone
 - Cefiderocol can be considered
 - MBL
 - Only cefiderocol is relevant to test
-

Testing of *P. aeruginosa*

- MBL: Only cefiderocol is relevant to test
 - Chromosomal mechanisms:
 - Ceftolozane-tazobactam and imipenem-relebactam generally of highest relevance
 - Depending on epidemiology, ceftazidime-avibactam and meropenem-vaborbactam may be relevant to test occasionally (class A carbapenemases)
-

Testing of *A. baumannii*

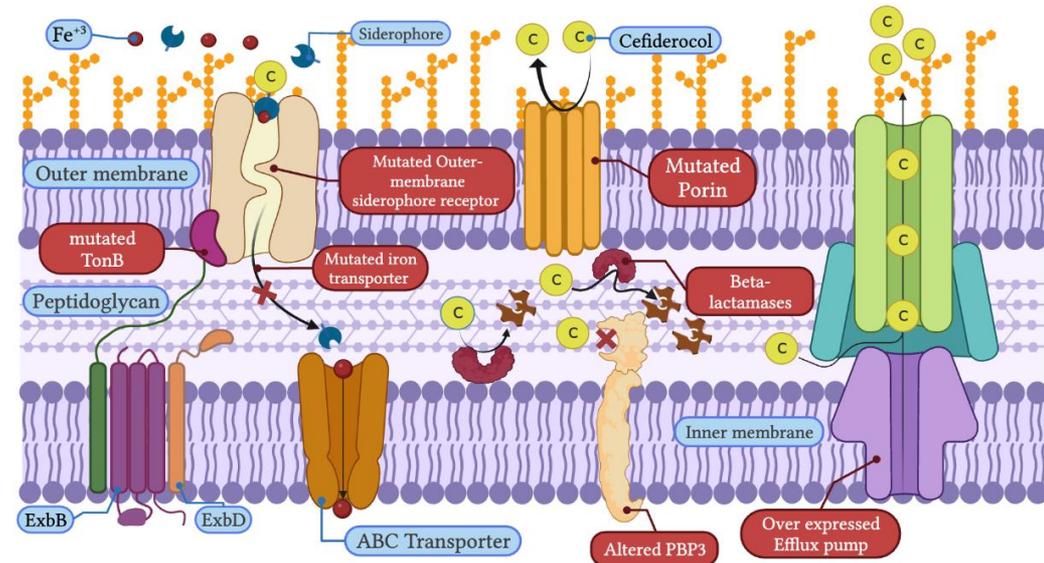
- Only cefiderocol has good in vitro activity
 - Ampicillin-sulbactam: carbapenemase-producers are often resistant (noteworthy: you need to test sulbactam alone – not fixed concentration of sulbactam....)
-

Emerging resistance



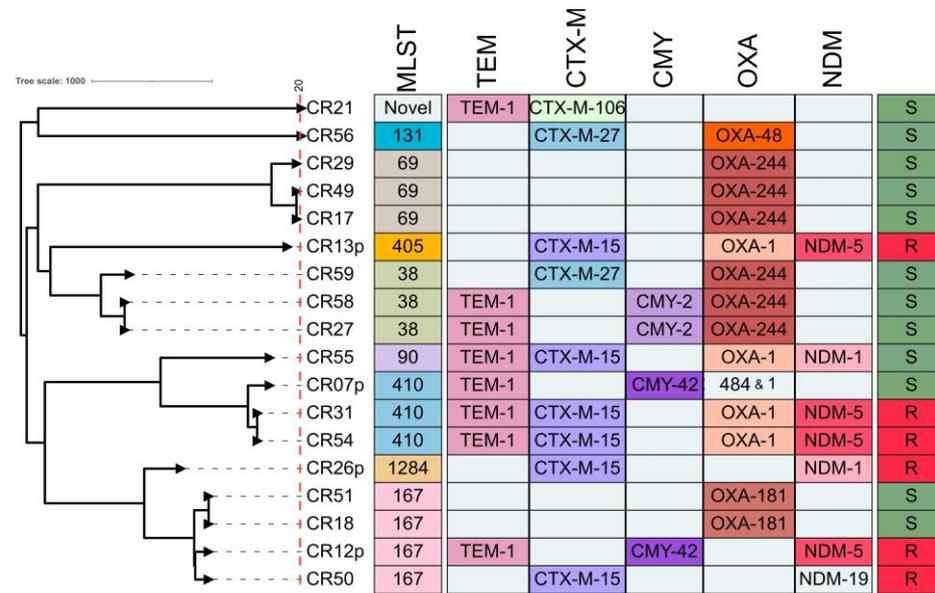
Resistance to cefiderocol

- Reduced permeability through deletion/mutation of:
 - Iron transporter (*fiu*)
 - Siderophore receptor (*cirA*)
 - Porins mutations (*ompK*, *ompC*)
 - Overexpression of efflux pumps
- Target modification
 - Mutations in PBP3
- Antibiotic modification
 - Presence of different beta-lactamases including NDMs, KPC-3 variants, OXA-427, and PER



Cefiderocol resistance (Stockholm)

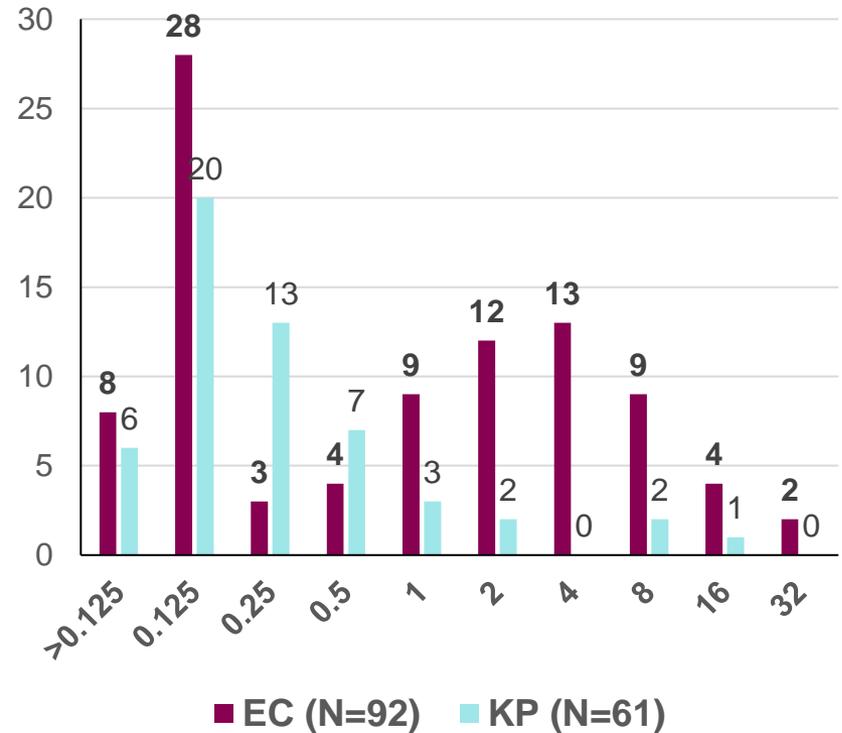
- 18 CP *Escherichia coli* were obtained.
- Disk diffusion method was employed to determine susceptibility
→ R: zone diameter < 22 mm
- Isolates were compared to an ATCC strains
- More than 92,000 non-synonymous mutations were identified between resistant and susceptible strains
- Currently challenging with BMD



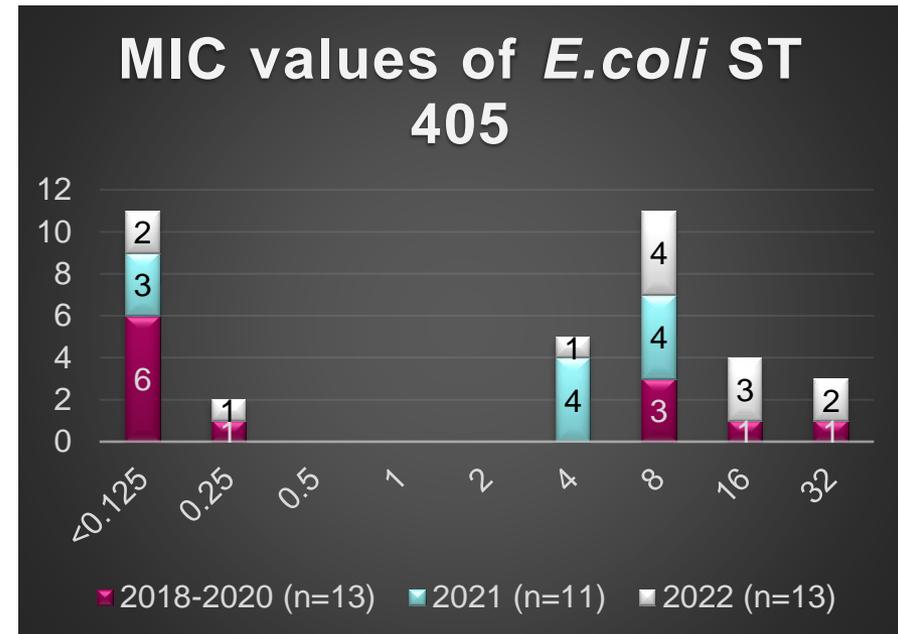
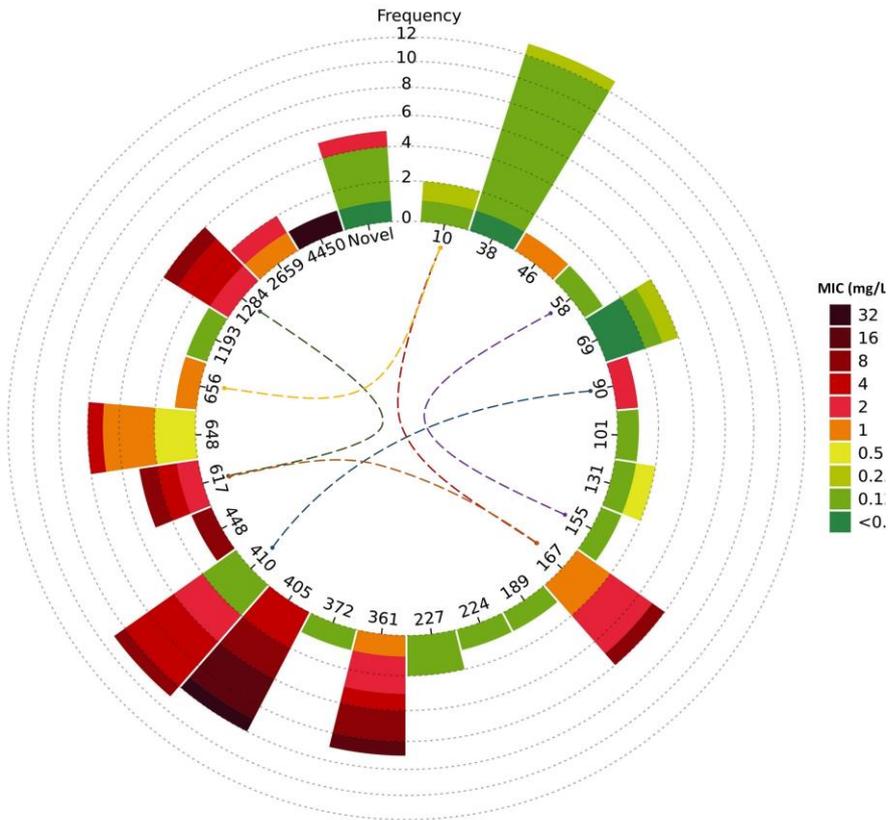
Strong correlation between resistance to CFDC and the presence of the *bla*_{NDM} gene

Aztreonam-avibactam resistance

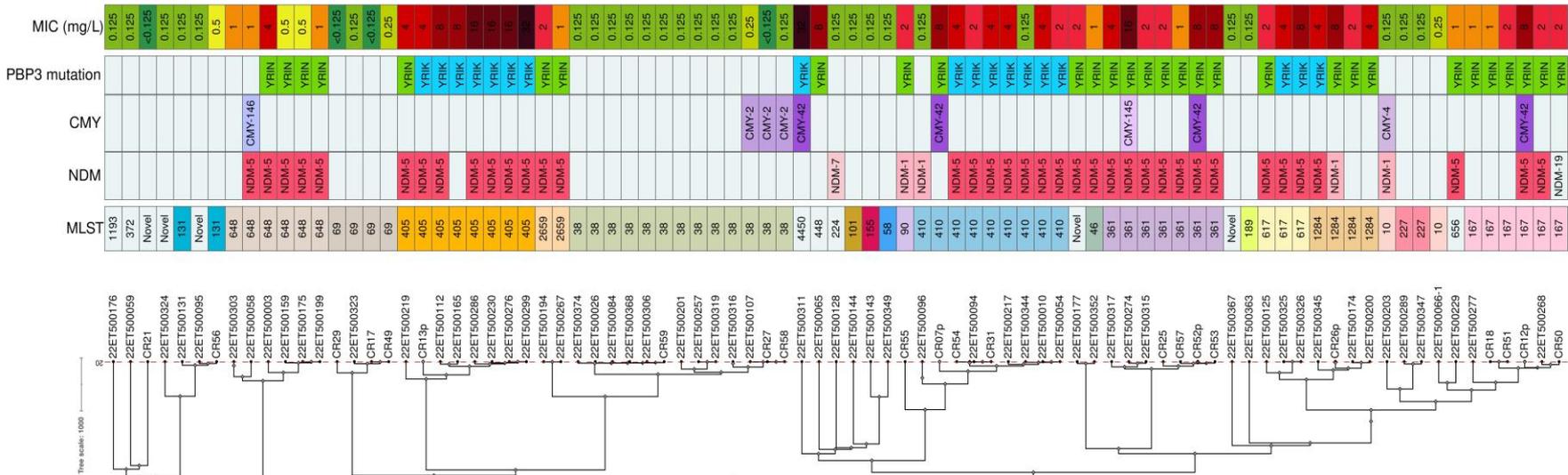
- Broth microdilution method with fixed concentration of AVI at 4mg/L
- At present, clinical breakpoints for ATM-AVI are not established
- Decreased susceptibility- when MIC \geq 4mg/L in most of the *in vitro* studies
- Breakpoint for aztreonam alone: S \leq 1 mg/L, R $>$ 4 mg/L
- MIC₉₀ for MBL-producing Enterobacterales was reported as 1 mg/L (<https://doi.org/10.1128/AAC.00472-17>)



Resistance common in *E. coli* ST405



NDM-5 and PBP-3 insertions – common in resistant strains



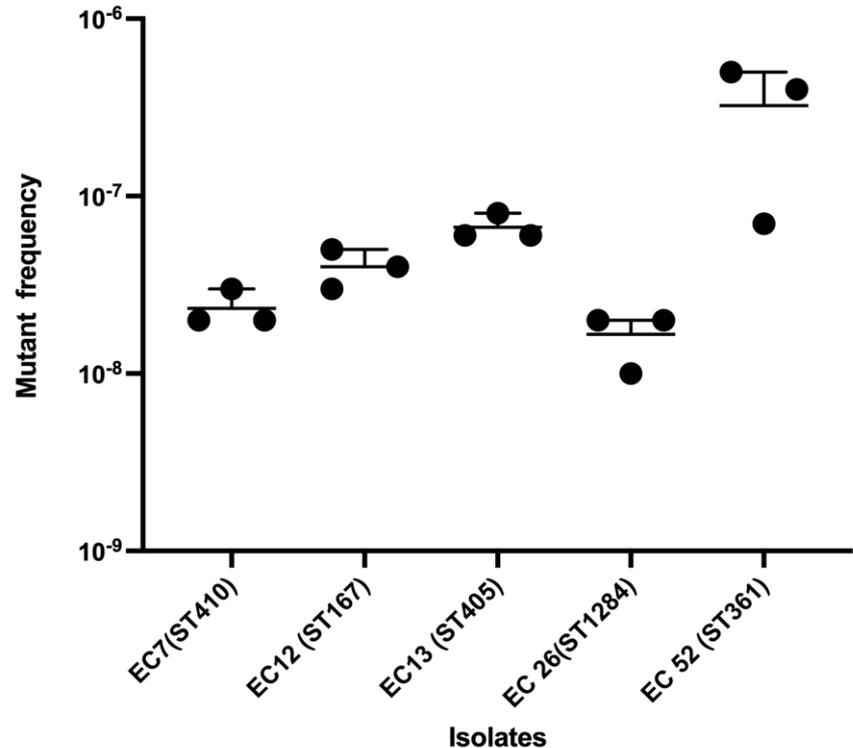
Dendrogram generated from cgMLST. Lower MICs in STs 648 and 167, despite the presence of NDM-5 and YRIN insertions

Presence of CMY not correlated with MIC-values

Potential relation to NDM-5 expression or other unknown factors

In vitro selection AZT-AVI: mutation frequency ranging between 10^{-8} to 10^{-7}

- Single step selection experiments were performed
- 29 EC and 19 KP isolates were tested
- MHA plates containing 2, 4, 8, 16 and 32 mg/L of ATM-AVI were used
- None of the KP isolates produced isogenic mutants
- Four NDM producers and one OXA-181 producer
- Mutation rates are similar to what has been observed in *Enterobacter* when exposed to cephalosporins (Kohlmann K et al. JAC 2018)



Some concluding remarks

- Knowledge about beta-lactamases will help guide testing – both indications and contraindications
 - If you do not know the beta-lactamase before testing it will still work to test all antimicrobials and let the breakpoints do the job
 - One exception might be OXA-48-group enzymes, where ceftazidime-avibactam may be the best treatment option
 - With disk diffusion you can test most of the new antimicrobials, although sometimes the result will not be conclusive (ATU)
 - Emerging resistance includes complex resistance mechanisms that cannot be detected with more simplistic approaches (presence/absence of beta-lactamases)
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Thank you!



Karolinska Institutet, South



Karolinska University Hospital