Challenges in AMR *K. pneumoniae* infections and treatment,

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Utstein Kloster, 3 May 2018

Research group

Department of Laboratory Medicine

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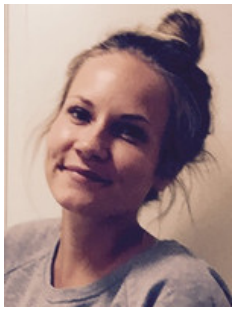





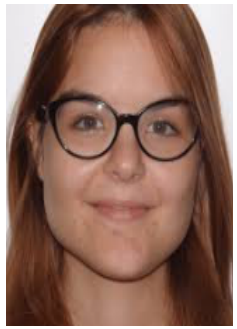


http://ki.se/en/labmed/christian-giske-group

Funding

Vetenskapsrådet

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jpiamr
JOINT PROGRAMMING INITIATIVE ON ANTIMICROBIAL RESISTANCE

Sida

Svenska Läkaresällskapet

When does antimicrobial resistance really matter?

- In severe disease: as a cause of mortality
  - → Frequently difficult to ascertain contribution to death
  - → Patient comorbidities can be a significant bias
  - → Some types of resistance come with greatly reduced fitness
  - → The role of strain factors usually insufficiently studied
- In other diseases: only when using “soft parameters”
  - → Length of stay/increased costs
  - → Impact on strain transmission
  - → Long-term consequences

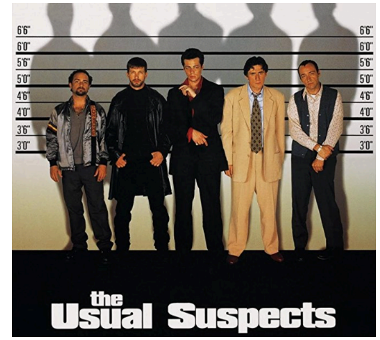
- Bloodstream infections: undisputable relevance, frequently access to strains, easier to compare between countries

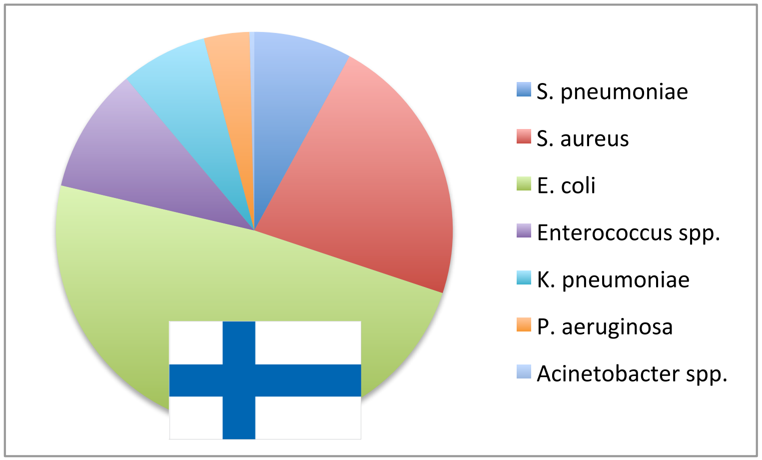
In vitro resistance vs clinical response

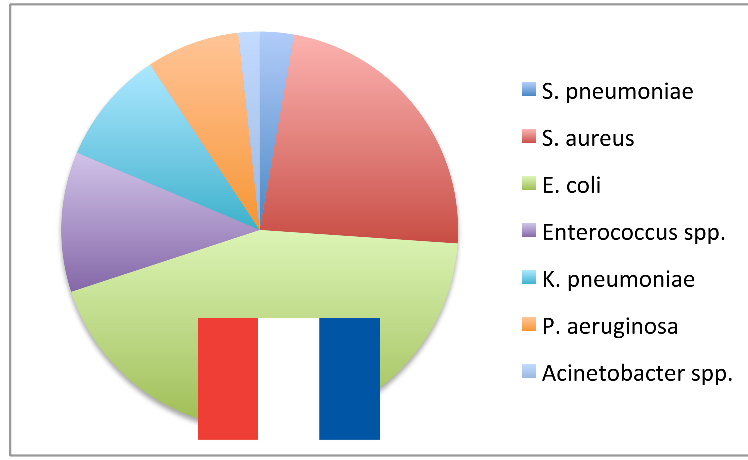
- Rex and Pfaller 2002 (antifungal AST)
  - → Susceptible=responds in 90% of the cases
  - → Resistant=still responds in around 60% of the cases
  - → Pertains to immunocompetent individuals with monomicrobial infections with predictable penetration of the drug to the infection site
  - → Polymicrobial infections with unpredictable penetration: even lower
- Strain variations
  - → Are usually major in preclinical PK-PD data
  - → Strain virulence is not taken into account in AST
  - → We also do not take into account individual variation in the immune system

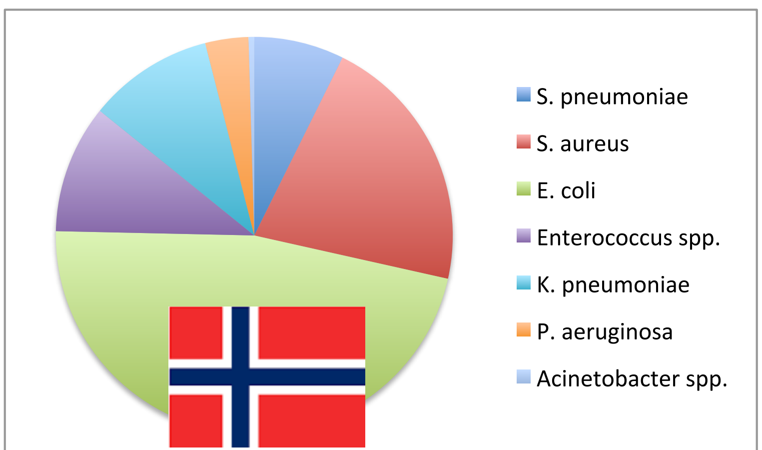
Doern GV and Brecher SM. JCM 2012

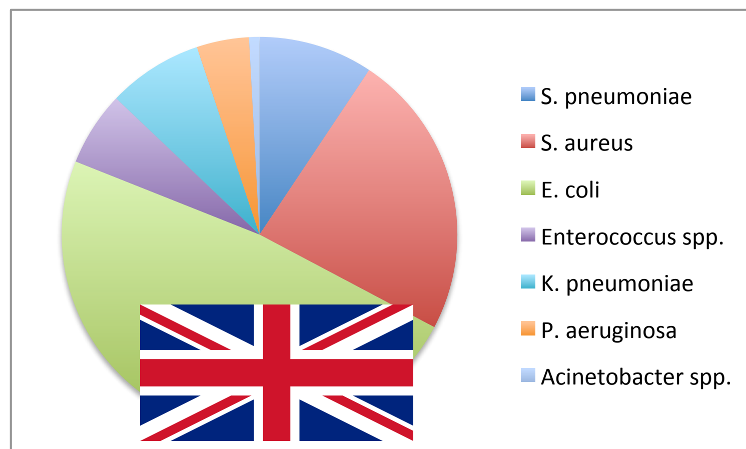
Bloodstream infections:





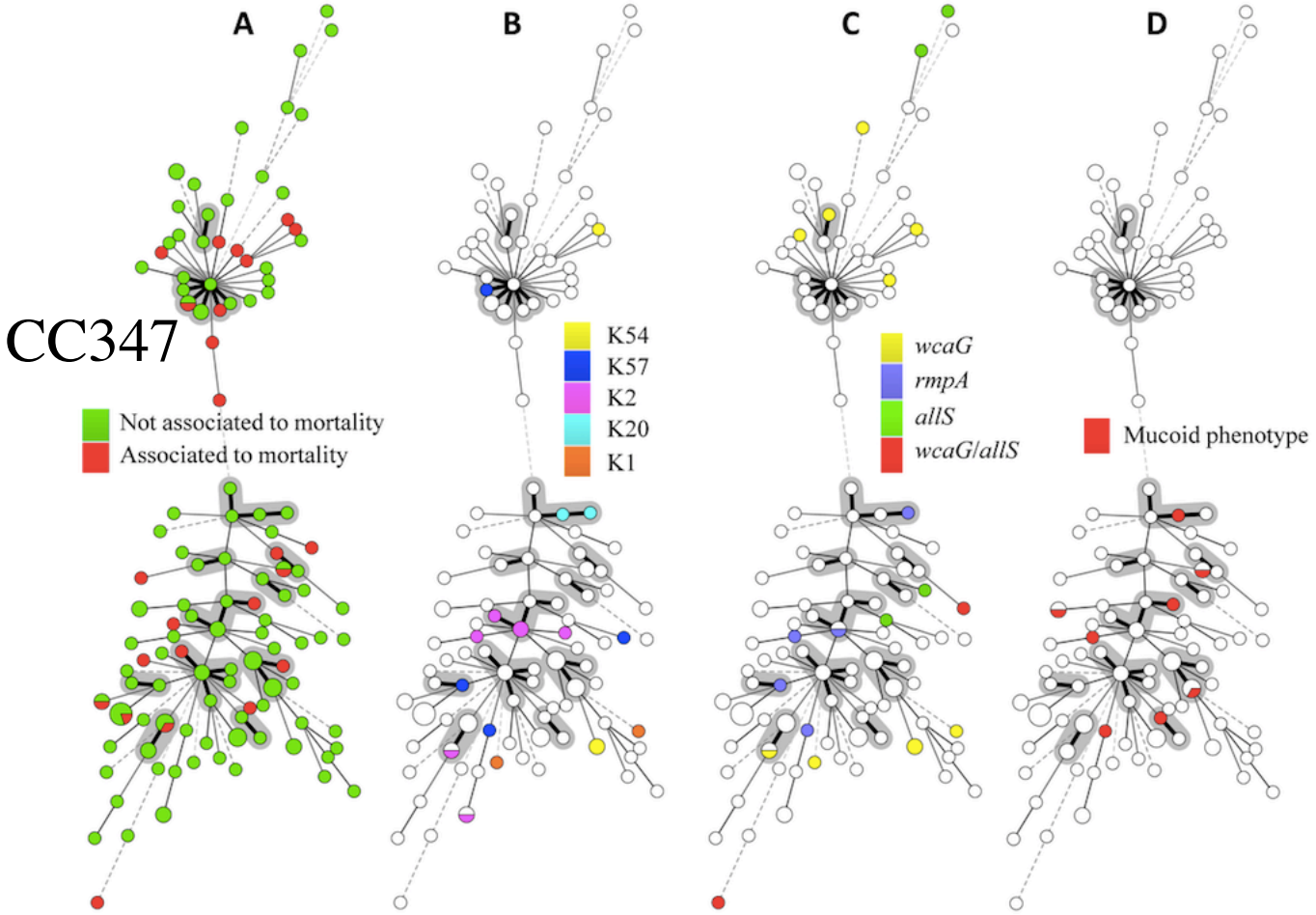






Klebsiella pneumoniae – invasive infections

K. pneumoniae (sensu latu), invasive infections Stockholm



Maatallah M et al. PloS One 2014. ST380, K2, *rmpA* was encountered, but was not associated with a fatal outcome

K. pneumoniae (sensu latu), invasive infections Stockholm

Parameter	Dead within 30 d	Survivors	P-value
Charlson (median)	5	2	0.0003
Metastatic cancer	43.5%	11.2%	0.001
KpIII (*variicola*)	43.5%	22.4%	

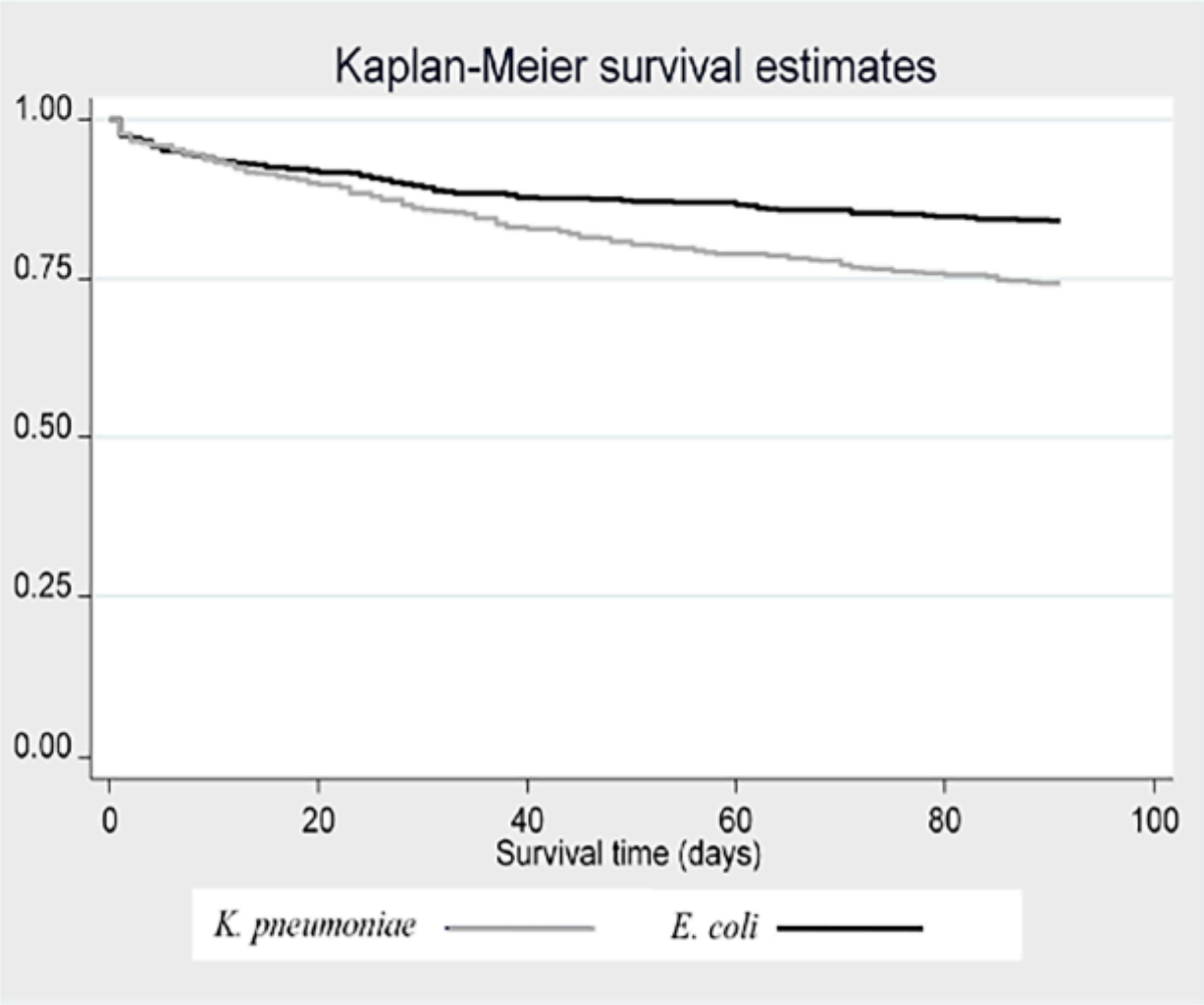
Invasive infection of *K. pneumoniae* vs *E. coli*

Table 1. Clinical characteristics of patients with invasive infection caused by *K. pneumoniae* versus *E. coli*, multivariable analysis.

	K. pneumoniae	*E. coli*	Adjusted odds
	n = 599	*n = 599*	ratio
Patients factors	No (%)	*No (%)*	(95% CI)
			K. pneumoniae vs
			E. coli
Peripheral vascular disease	30 (5)	14 (2)	3.74 (1.65–8.48)
COPD	58 (10)	37 (6)	1.96 (1.14–3.36)
Kidney disease	105 (18)	69 (12)	1.90 (1.28–2.82)
Bile disease	36 (6)	15 (3)	3.10 (1.44–6.66)
Hematological malignancy	112 (19)	76 (13)	1.70 (1.07–2.70)
Bile/liver/pancreas malignancy	51 (9)	22 (4)	3.45 (1.77–6.75)
Colorectal malignancy	42 (7)	24 (4)	2.56 (1.34–4.89)
Urinary catheter	191 (32)	111 (19)	2.36 (1.64–3.40)
Central catheter	190 (32)	96 (16)	2.32 (1.53–3.54)
Hospital-acquired[a)]	178 (30)	197 (33)	0.53 (0.37–0.77)
Healthcare- associated community-onset[a)]	163 (27)	55 (9)	3.06 (2.03–4.62)

Vading M et al. PloS One 2018

Higher 30-d mortality in *K. pneumoniae*



P<0.001

Factors associated with mortality

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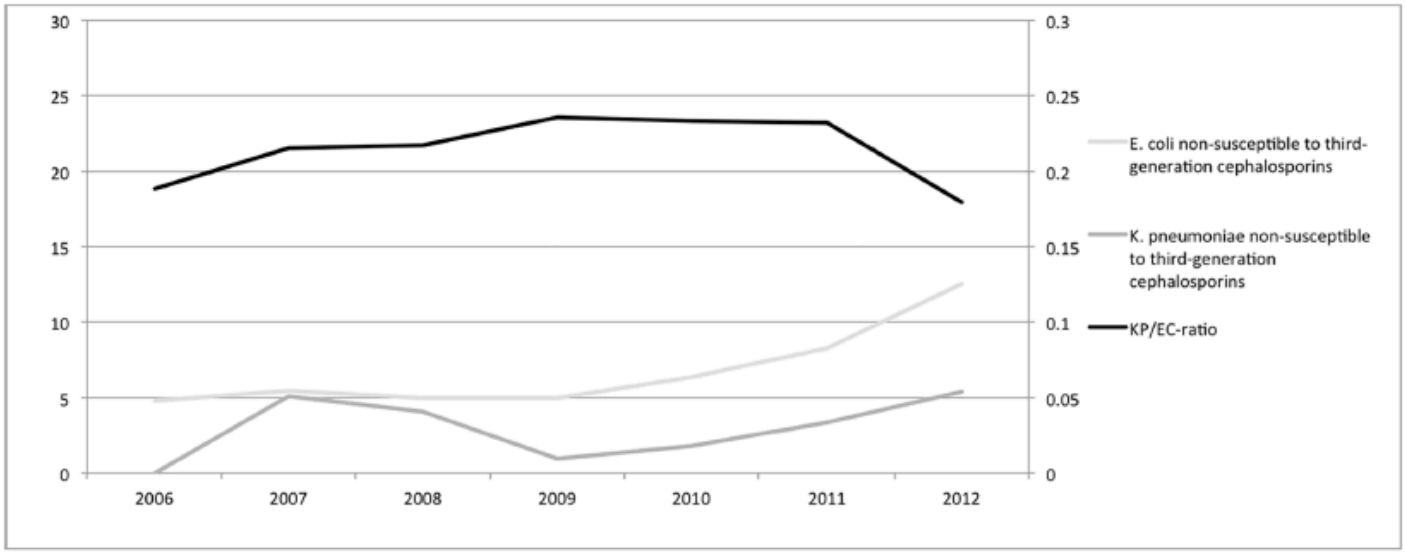
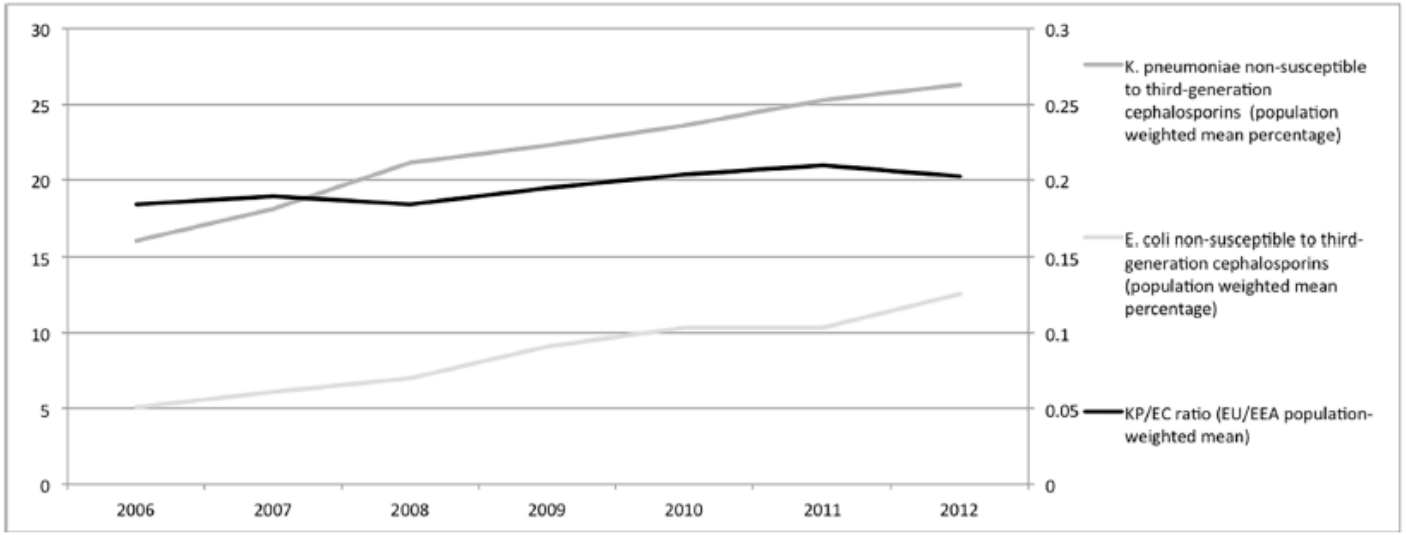
Table 2. Associated factors for mortality in the extended *K. pneumoniae* cohort, factors significant in multivariable analysis.

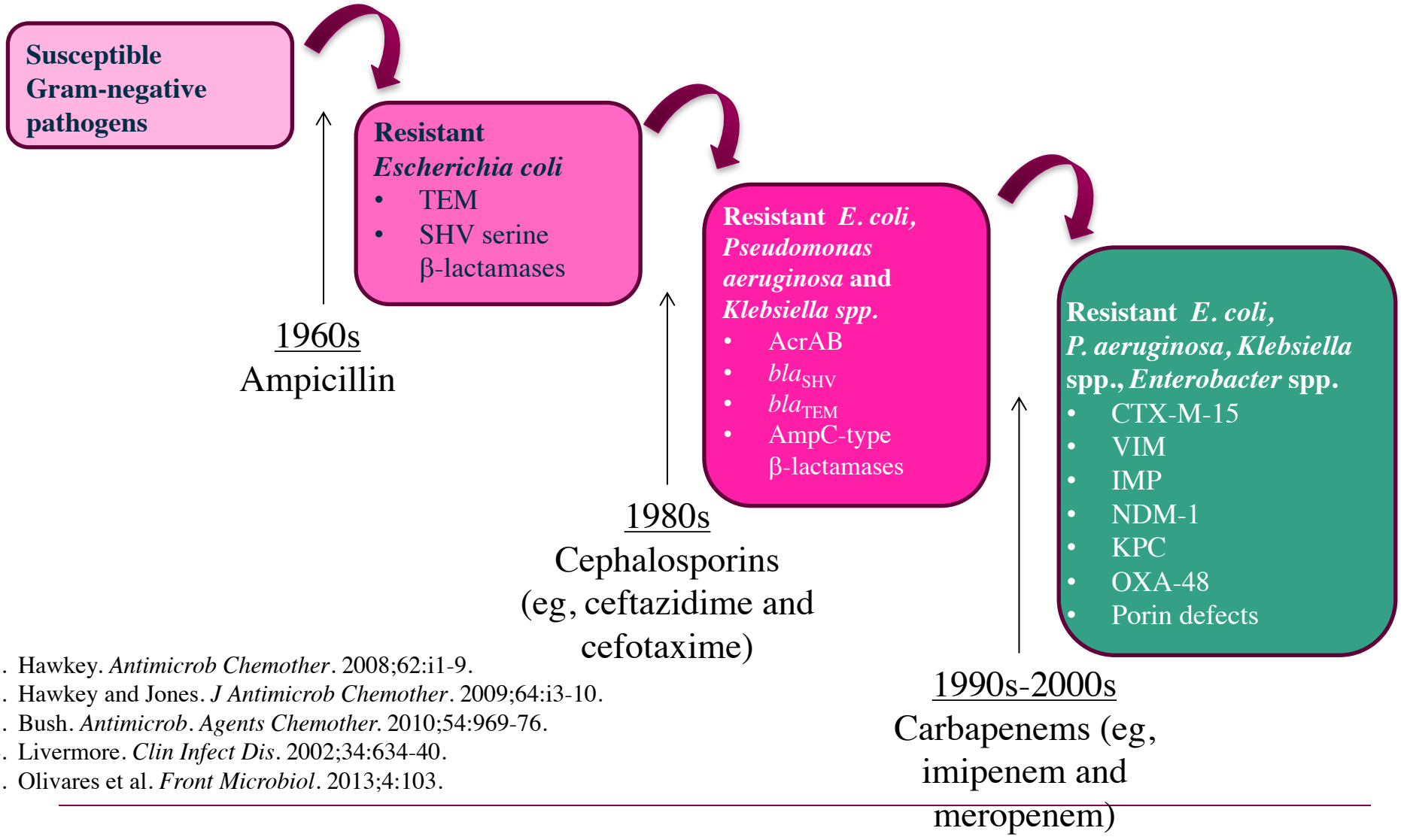
	Mortality within 7d	Mortality within 30 d	Mortality within 90 d
*Associated factors**	(n = 43)	(n = 101)	(n = 176)
	Adjusted OR	Adjusted OR	Adjusted OR
Age	1.03 (1.00–1.05)	1.02 (1.00–1.04)	1.03 (1.01–1.04)
Polymicrobial infection	3.07 (1.51–6.27)	2.20 (1.32–3.68)	
Kidney disease			2.33 (1.41–3.84)
CNS disease		3.12 (1.73–5.62)	2.09 (1.26–3.45)
Lung malignancy	13.45 (3.94–45.90)	13.20 (4.11–42.38)	20.77 (6.01–71.73)
Urogenital, GI, bile/liver/pancreas malignancy		2.07 (1.10–3.91)	3.07 (1.87–5.05)
Hematological malignancy		3.13 (1.47–6.63)	2.50 (1.33–4.72)
Other malignancy**	6.18 (1.87–20.41)	6.34 (2.54–15.85)	3.77 (1.63–8.74)
Hospital-acquired			1.99 (1.21–3.28)
Healthcare-associated community-onset			1.69 (1.02–2.79)
Source of infection			
Respiratory tract	3.62 (1.01–13.04)	3.79 (1.32–10.87)	3.74 (1.44–9.68)
Bile/liver, GI		1.92 (1.00–4.16)	1.91 (1.15–3.15)
Unknown		2.09 (1.05–4.16)	

K. pneumoniae: expanding pathogen in Europe, not in Sweden



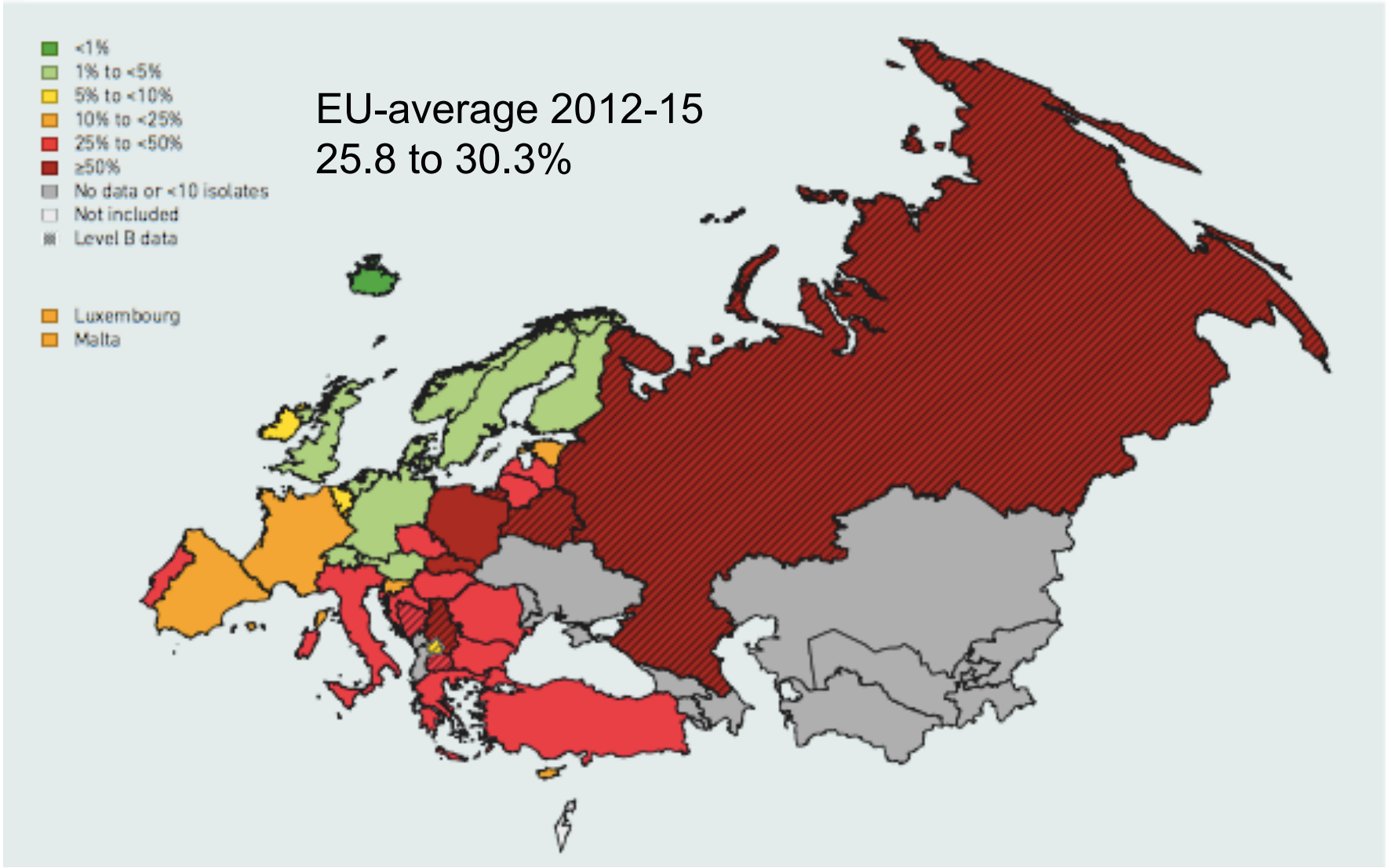


Evolution of Gram-negative Resistance

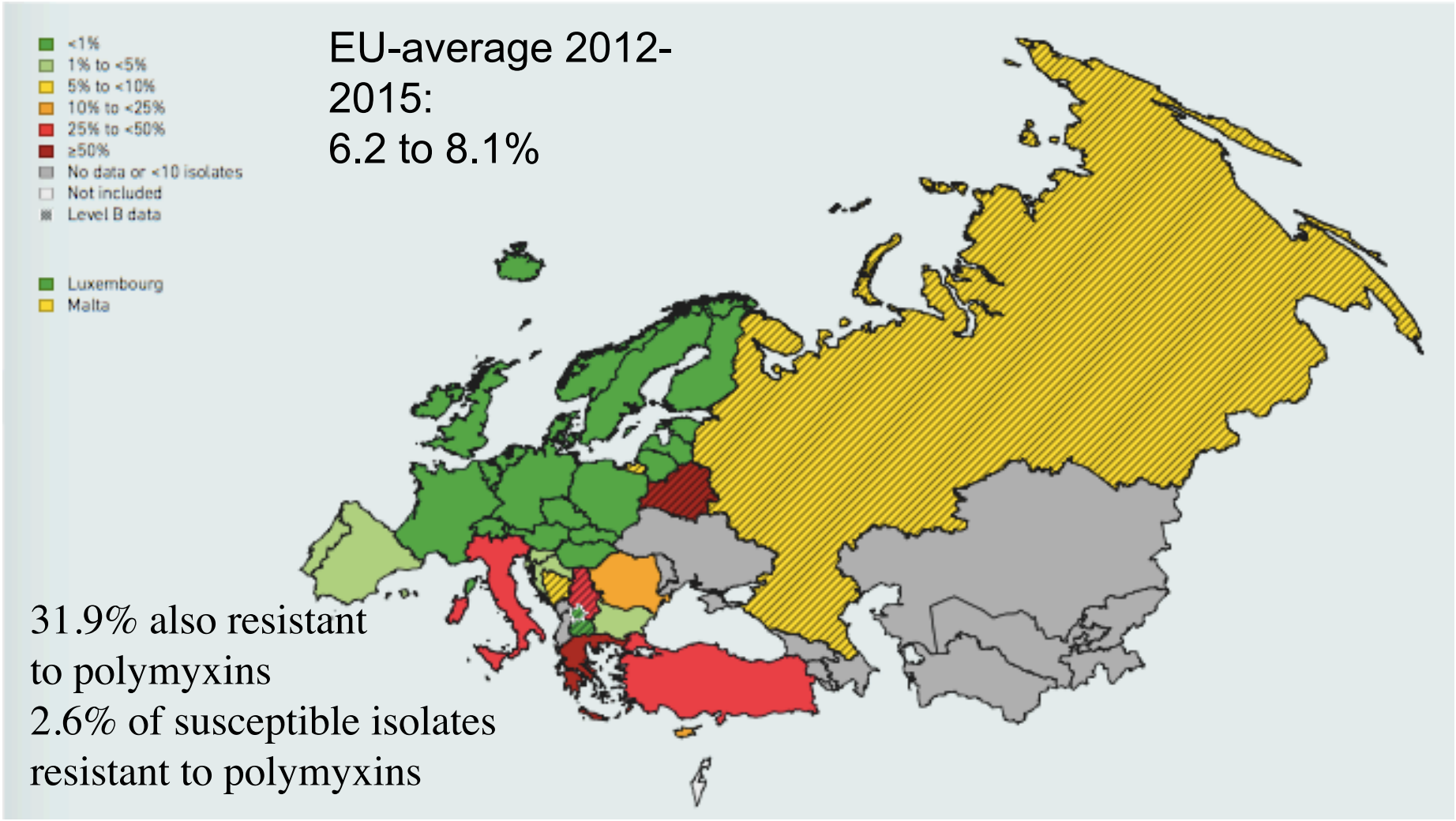


1. Hawkey. *Antimicrob Chemother*. 2008;62:i1-9.
2. Hawkey and Jones. *J Antimicrob Chemother*. 2009;64:i3-10.
3. Bush. *Antimicrob. Agents Chemother.* 2010;54:969-76.
4. Livermore. *Clin Infect Dis*. 2002;34:634-40.
5. Olivares et al. *Front Microbiol*. 2013;4:103.

ESBL-producing *K. pneumoniae*



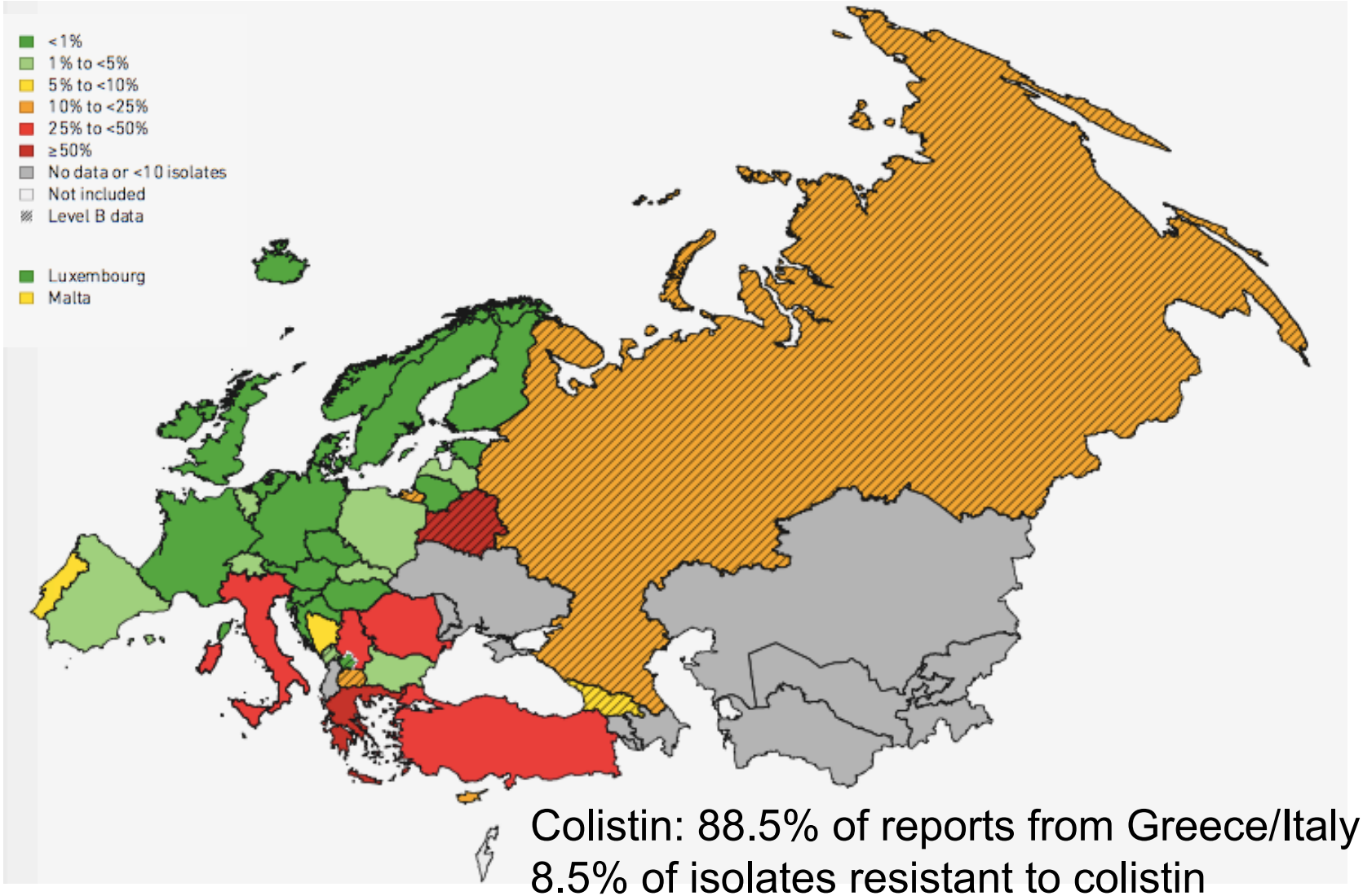
Carbapenem resistant *K. pneumoniae*



EU-average 2012-2015:
6.2 to 8.1%

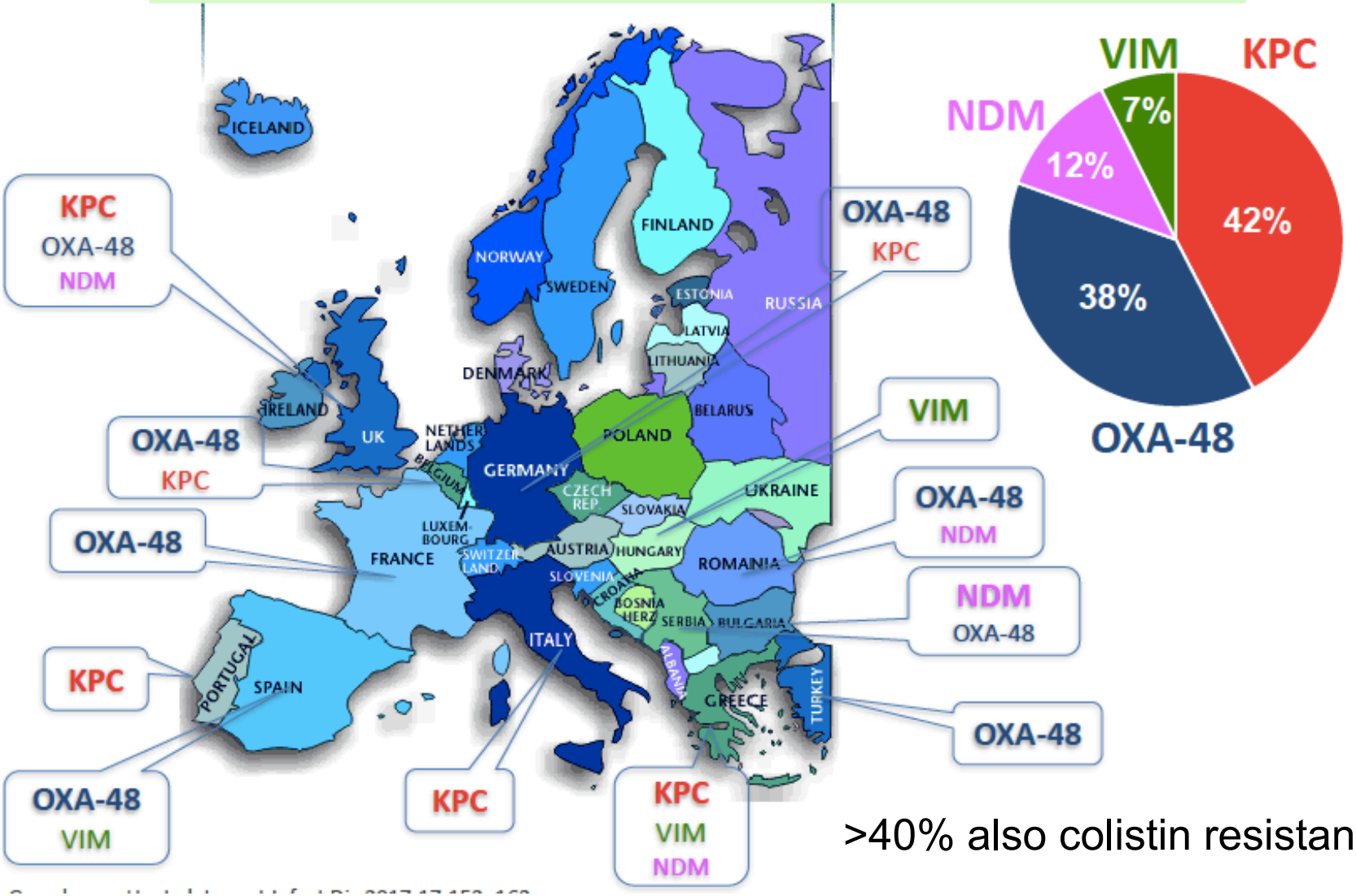
31.9% also resistant to polymyxins
2.6% of susceptible isolates resistant to polymyxins

Carbapenem-R *K. pneumoniae*, 2016



Colistin: 88.5% of reports from Greece/Italy
8.5% of isolates resistant to colistin

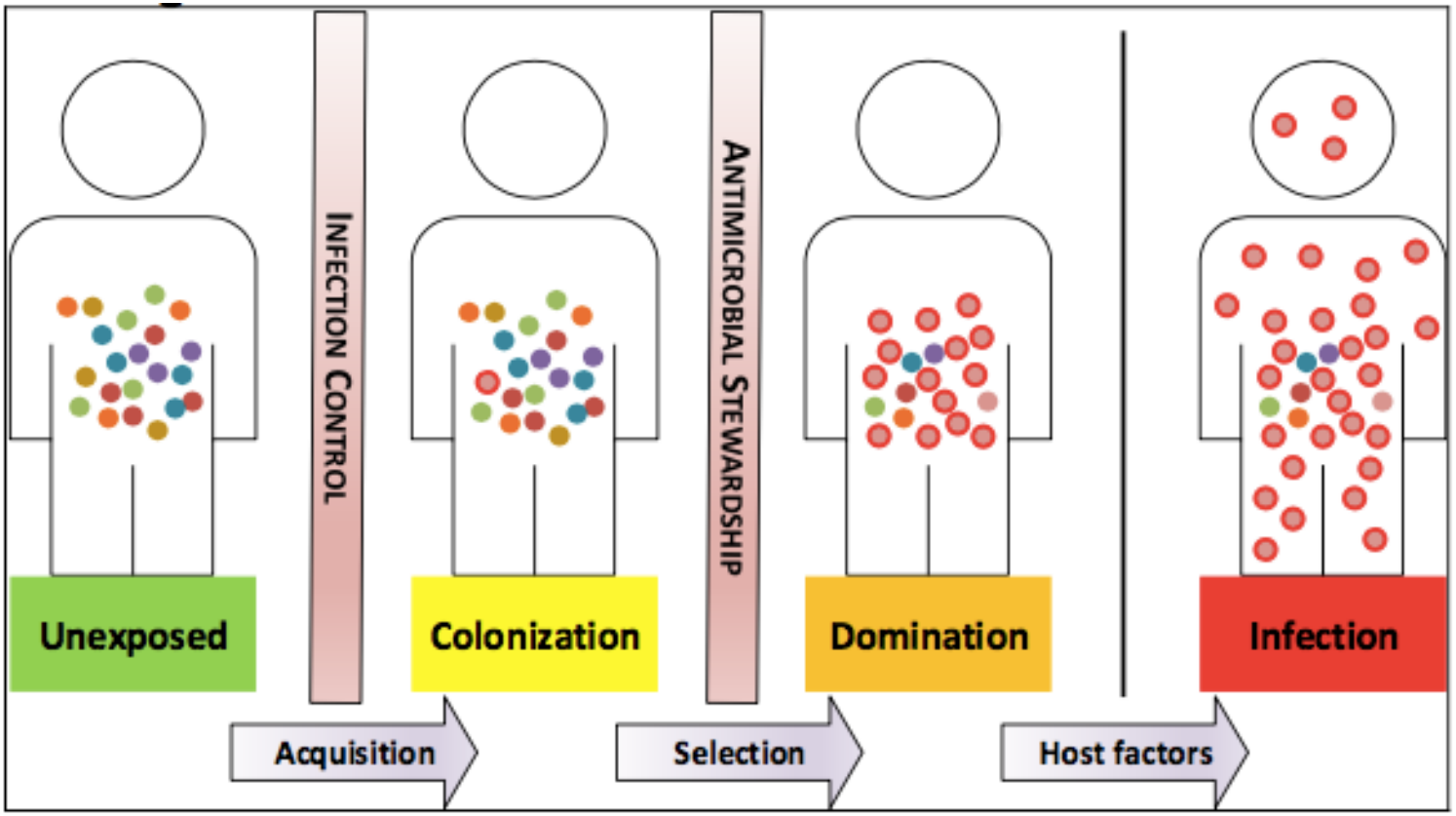
CPE genotypes – situation in Europe



>40% also colistin resistant

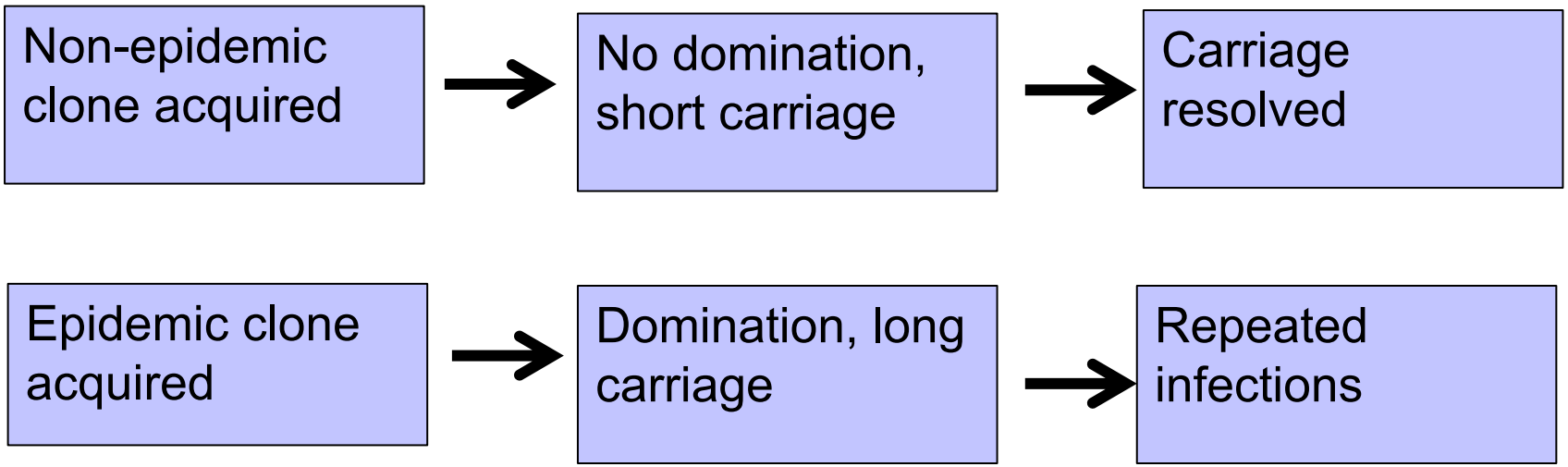
Grundmann H, et al. Lancet Infect Dis. 2017;17:153–163.

The process from colonization to infection



Hypothesis under investigation in recently funded multicentre study (Joint Programming Initiative AMR, JPIAMR)

Colonization to infection – a hypothesis



- Process likely influenced by antimicrobial consumption
- We have the tools to stratify in high-risk and low-risk carriage
- Therapeutic interventions: directed towards high-risk carriage
- Effects could be monitored by continuous surveillance of clones occurring in bloodstream infection

Klebsiella pneumoniae – novel treatment

Activity of new drugs

Antimicrobial	EBA ESBL	EBA AmpC	KPC KP	EBA MBL	PA MDR	AB MDR	SM
Ceftolozane-tazobactam	+	-	-	-	+	-	-
Ceftazidime-avibactam	+	+	+	-	+/-	-	-
Meropenem-vaborbactam	+	+	+	-	+/-	-	-
Imipenem-relebactam	+	+	+	-	+/-	-	-
Cefiderocol	+	+	+	+	+	+	+
Plazomicin	+	+	+	+/-	+	+	-
Eravacycline	+(?)	+(?)	+(?)	+(?)	-	?	?
Colistin	+	+	+	+	+	+	+

Adapted from Falagas ME et al. Expert Review of Anti-infect. Ther. 2016; 8:747-763

Emerging resistance to new agents…

- *K. pneumoniae* ST258, bloodstream infection – isolates on day 1 (MIC 4 mg/L) and 2 (MIC 32 mg/L) (meropenem therapy)
- PacBio sequencing of both strains
- OmpK35 and K36 mutations
- Increase expression of KPC-3 (multiple copies of Tn4401 transposon containing KPC-3)

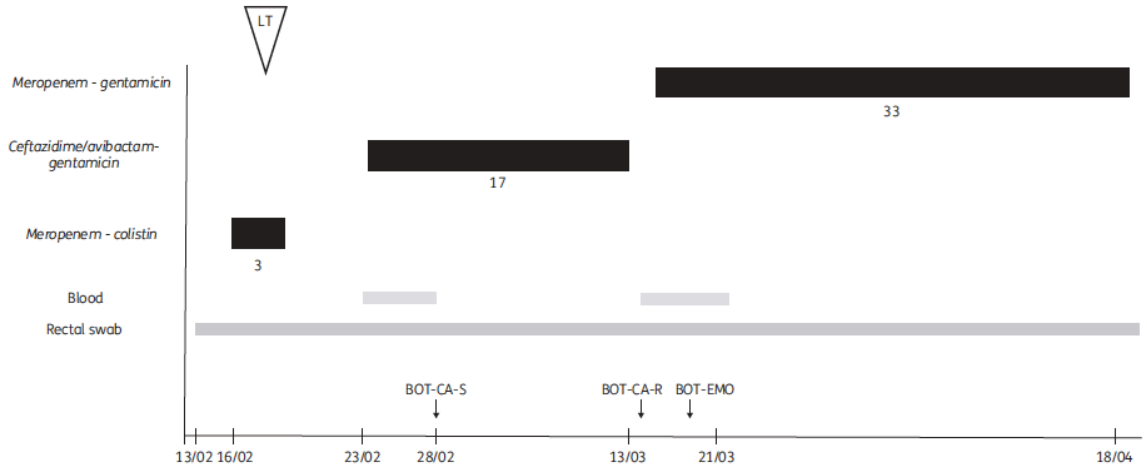
Resistance to Ceftazidime-Avibactam in *Klebsiella pneumoniae* Due to Porin Mutations and the Increased Expression of KPC-3

Romney M. Humphries, Peera Hemarajata
Pathology and Laboratory Medicine, University of California, Los Angeles, Los Angeles, California, USA

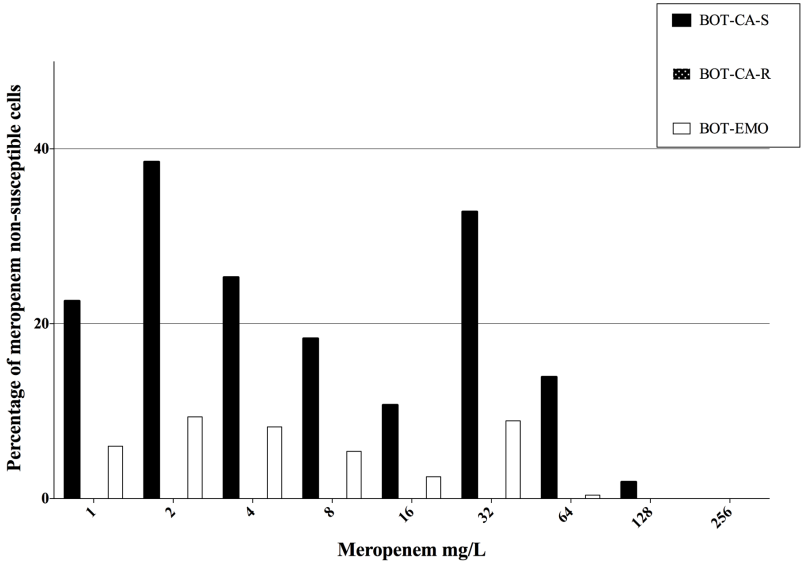
KEYWORDS ceftazidime-avibactam, KPC, OmpK35, OmpK36, resistance

AAC 2017; 61: e00537-17.

Heteroresistance to ceftazidime-avibactam



Gaibani P et al.
JAC 2018



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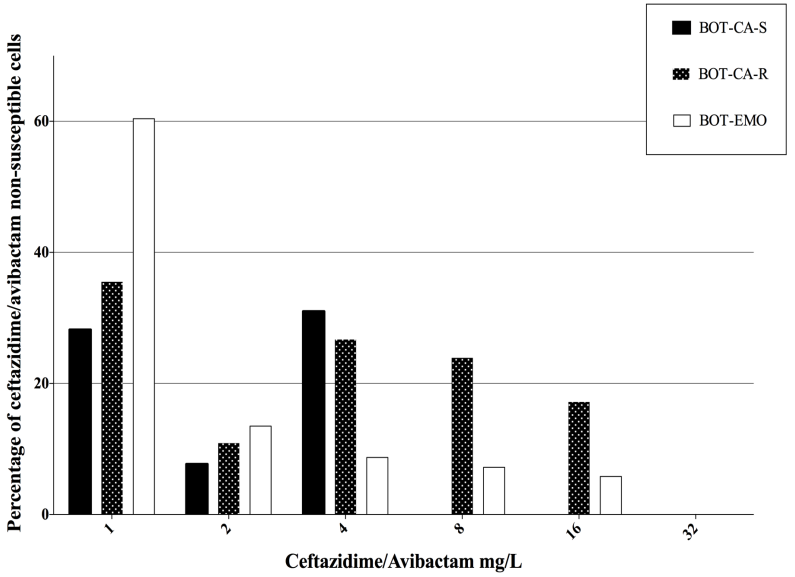
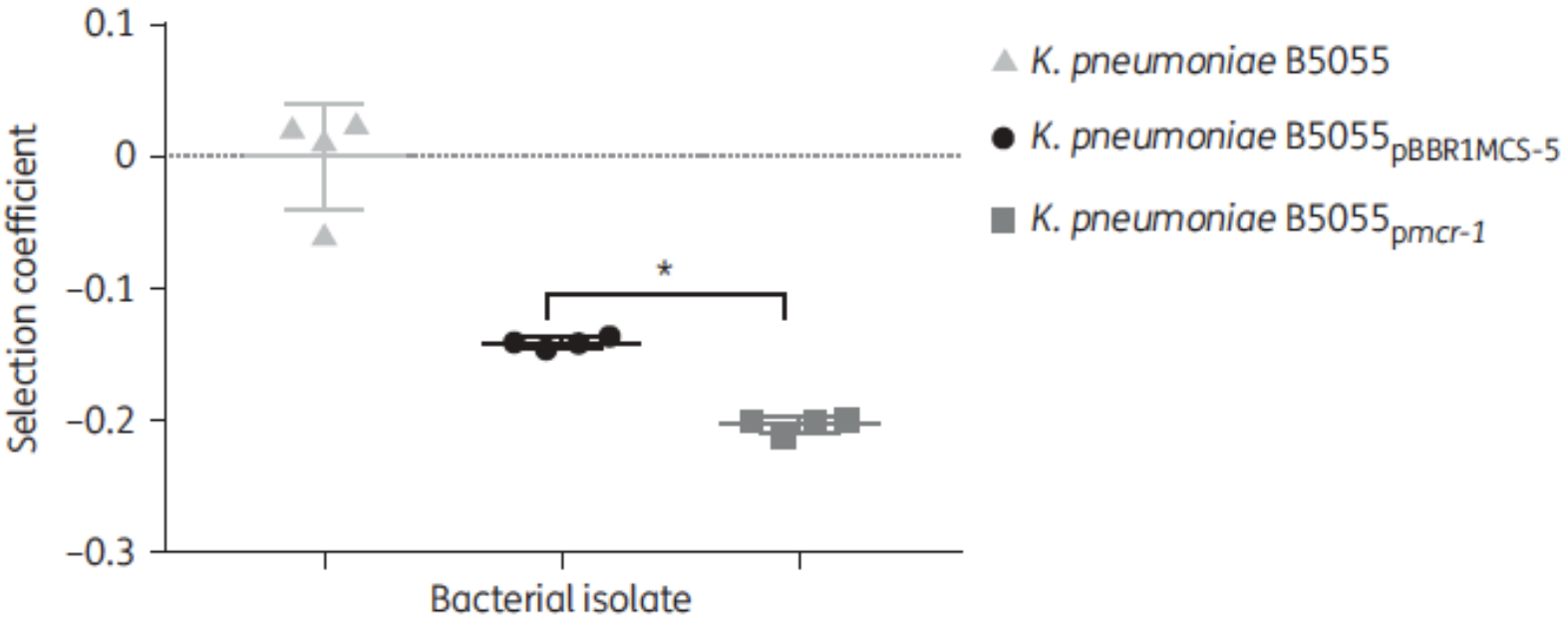


Mobile colistin resistance not the most common, but gets most attention…

MCR-type	Country	Species	Source
mcr-1	China	*E. coli*	Animal
mcr-1.2	Italy	*K. pneumoniae*	Human
mcr-1.3	China	*Salmonella*	Animal
mcr-2	Belgium	*E. coli*	Animal
mcr-3	China	*E. coli*	Animal
mcr-4	Italy	*Salmonella*	Animal
mcr-5	Germany	*Salmonella*	Animal

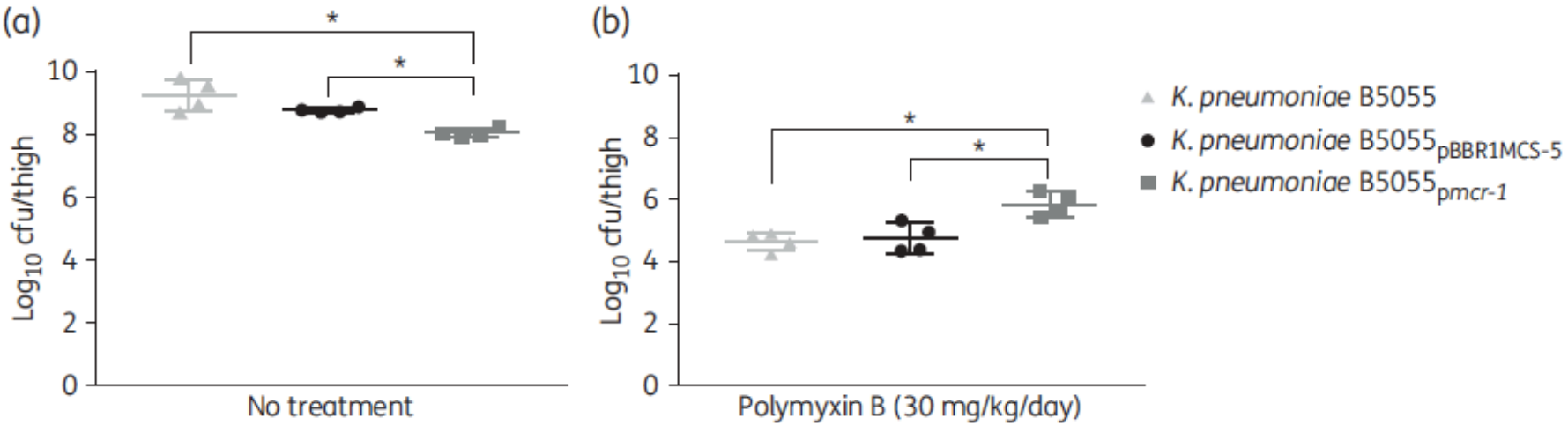
Liu et al. Lancet ID 2016; Xavier et al. Euro Surveill 2016;
Di Pilato et al. AAC 2016; Yin et al. mBio 2017;
Carattoli et al. Euro Surveill 2017; Borowiak et al. JAC 2017;
Lu et al. AAC 2017

Fitness cost of carrying *mcr-1*



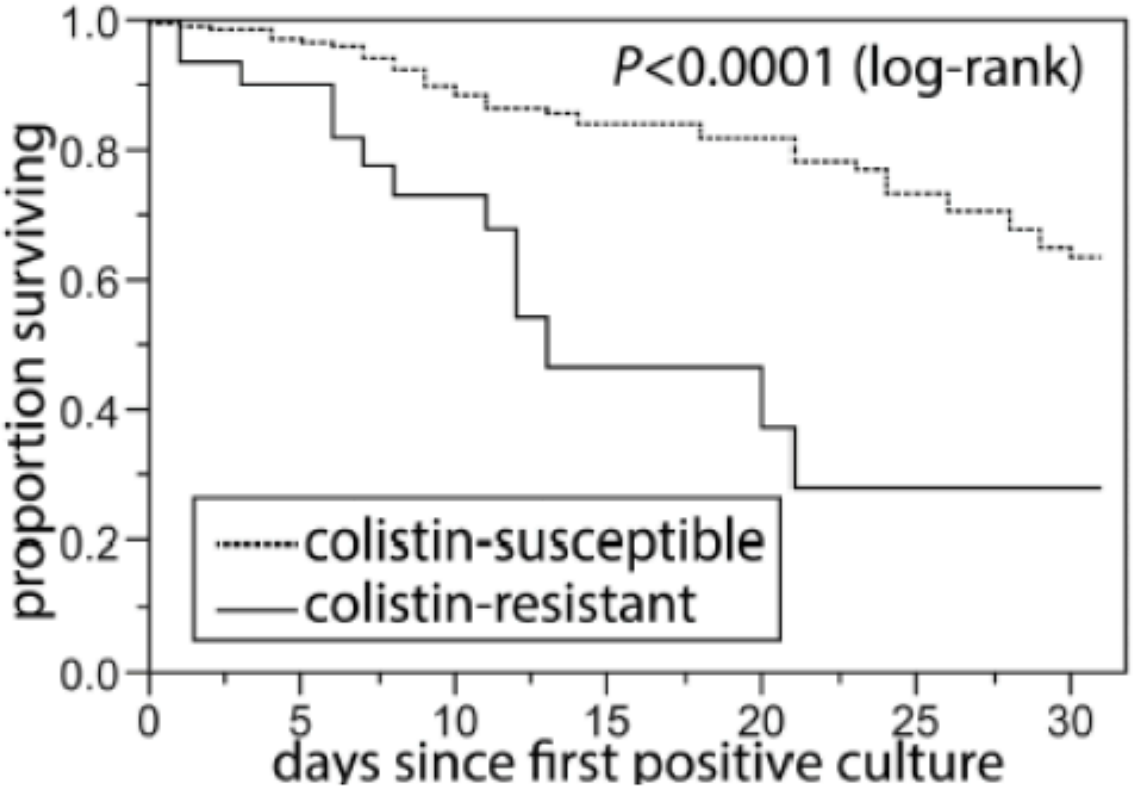
- In vitro competition assay (vs *E. coli* JW1)
- *K. pneumoniae* parental strain defined as selection coefficient zero
- Nang SC et al. JAC 2018

mcr-1 confers a selective advantage only under selective pressure with polymyxins



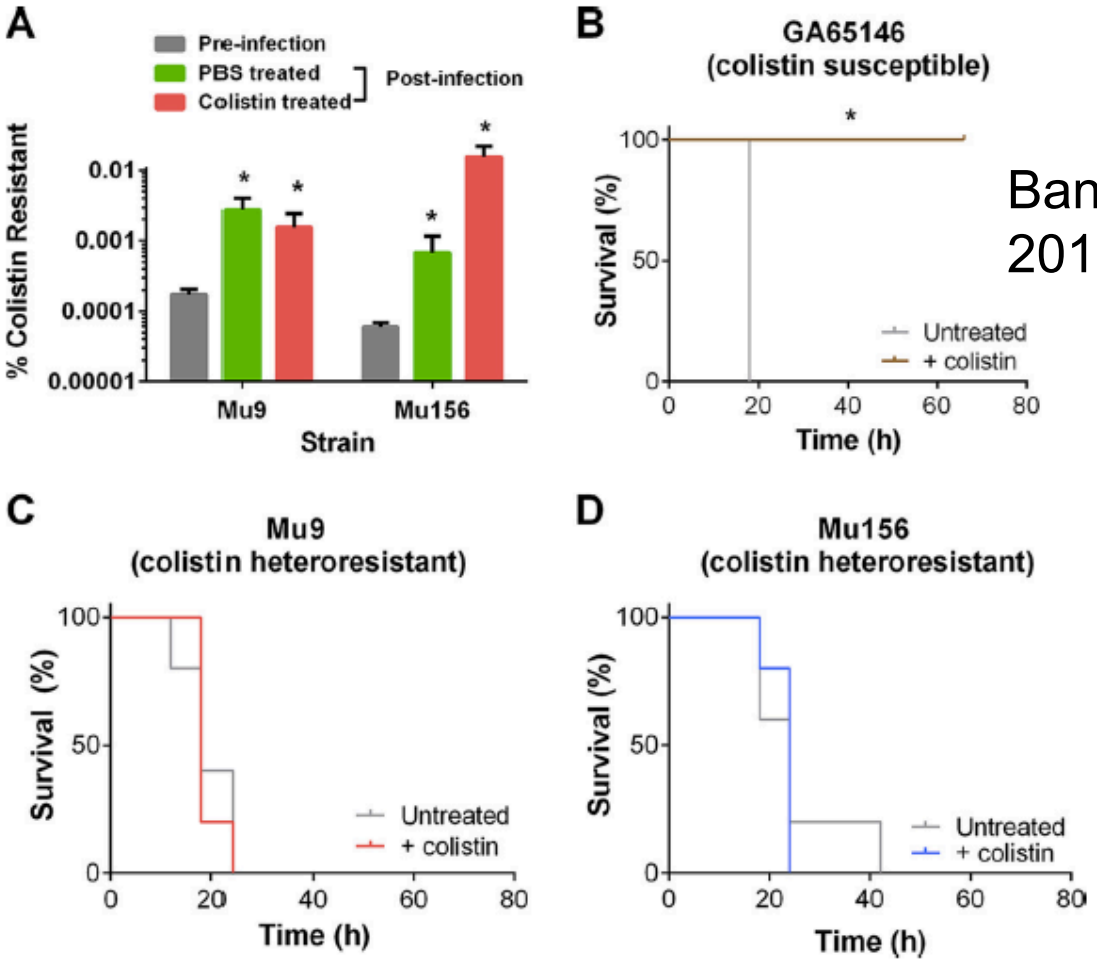
- Could indicate that e.g. carriage of *mcr-1* could have a low impact in individuals that are not exposed to polymyxins
- Consistent with the emergence of *mcr* in regions where polymyxin consumption is high

Colistin-resistant *K. pneumoniae* – survival in carbapenemase-producers



Rojas LR. CID 2017

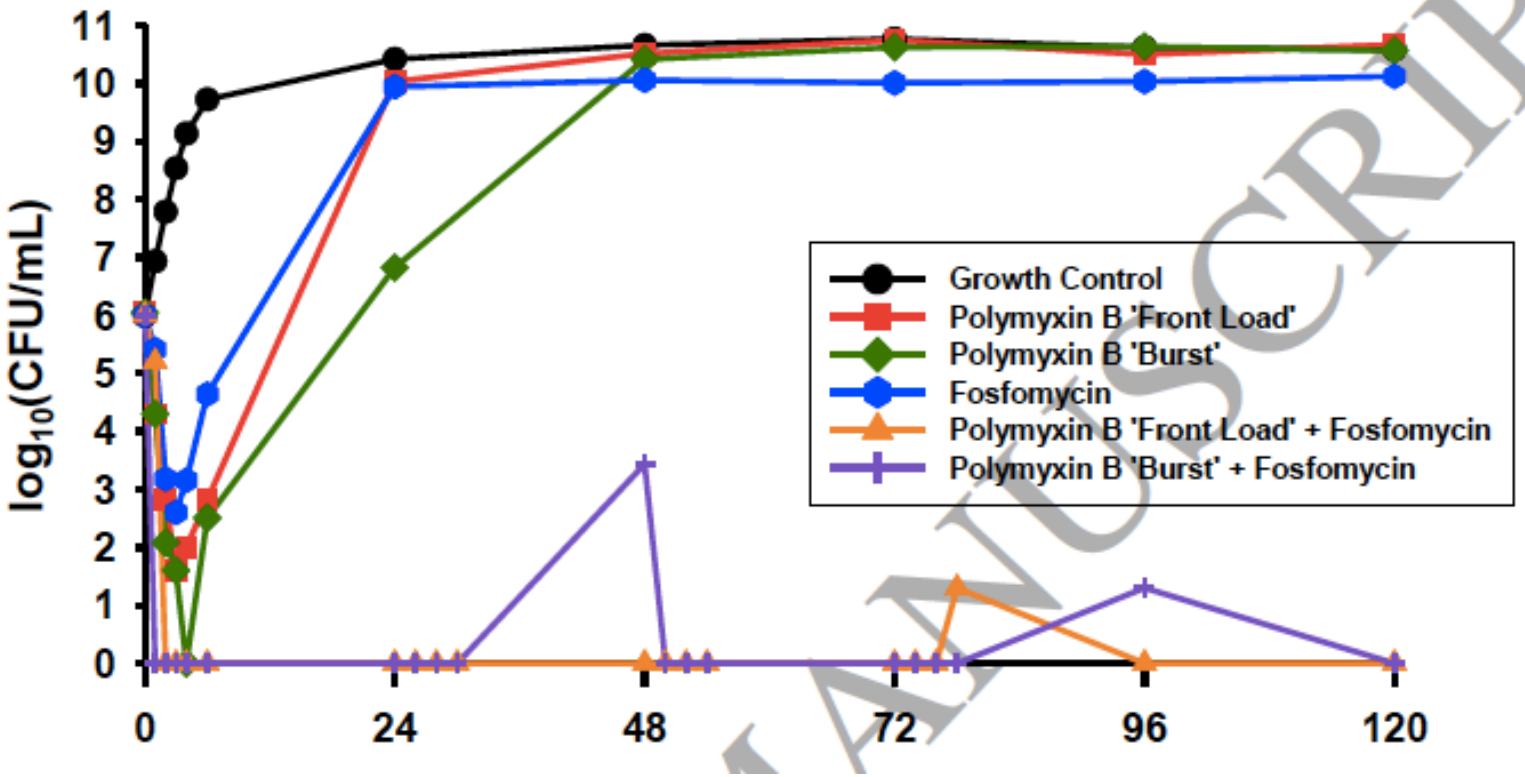
Colistin heteroresistance matters in the murine peritonitis model



Band VI, et al. mBio 2018; 9:e02448-17.

Hollow fiber: polymyxin B+fosfomycin prevents regrowth

Bulman ZP et al. IJAA 2018

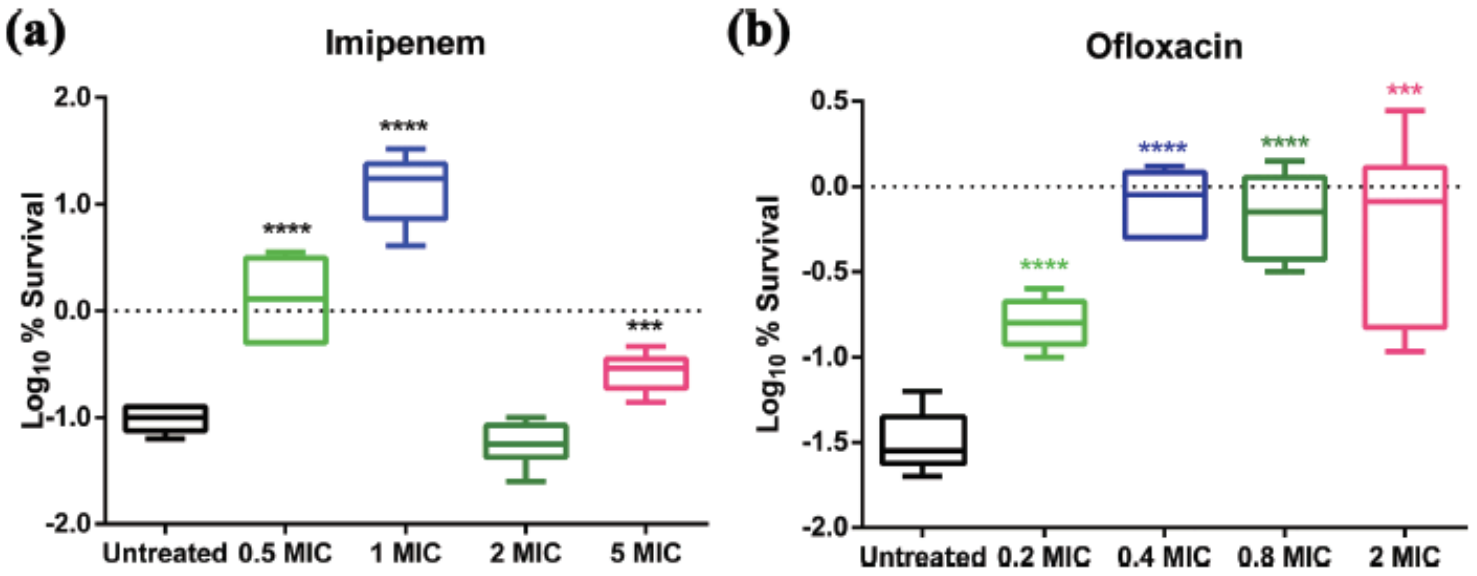


Pan-resistant *K. pneumoniae*

Class and antimicrobial(s)	MIC(s) (μg/ml)	Interpretation	Associated resistance gene(s)
Aminoglycoside			
Amikacin	>64	R	*aacA4*, *rmtC*
Gentamicin	>16	R	*aacA4*, *rmtC*
Tobramycin	>16	R	*aacA4*, *rmtC*
Beta-lactam			
Ampicillin	>32	R	$bla_{CTX-M-15}$, bla_{SHV-28}, bla_{CMY-6}, bla_{NDM-1}
Aztreonam	>64	R	$bla_{CTX-M-15}$, bla_{CMY-6}
Cefazolin	>8	R	$bla_{CTX-M-15}$, bla_{SHV-28}, bla_{CMY-6}, bla_{NDM-1}
Cefepime	>32	R	$bla_{CTX-M-15}$, bla_{NDM-1}
Cefotaxime	>64	R	$bla_{CTX-M-15}$, bla_{CMY-6}, bla_{NDM-1}
Cefotaxime-clavulanic acid	>32/4	ND	bla_{CMY-6}, bla_{NDM-1}
Cefoxitin	>16	R	bla_{CMY-6}, bla_{NDM-1}
Ceftazidime	>128	R	$bla_{CTX-M-15}$, bla_{CMY-6}, bla_{NDM-1}
Ceftazidime-avibactam[b]	>16/4	R	bla_{NDM-1}
Ceftazidime-clavulanic acid	>64/4	ND	bla_{CMY-6}, bla_{NDM-1}
Ceftriaxone	>32	R	$bla_{CTX-M-15}$, bla_{CMY-6}, bla_{NDM-1}
Doripenem	>8	R	bla_{NDM-1}
Ertapenem	>8	R	bla_{NDM-1}
Imipenem	32	R	bla_{NDM-1}
Meropenem	>8	R	bla_{NDM-1}
Piperacillin-tazobactam	>128/4	R	bla_{NDM-1}
Chloramphenicol			
Chloramphenicol	>16	R	Truncated *ramR*
Fluoroquinolone			
Ciprofloxacin	>8	R	*oqxA*, *oqxB*, *gyrA* and *parC* mutations, truncated *ramR*
Levofloxacin	>8	R	*oqxA*, *oqxB*, *gyrA* and *parC* mutations, truncated *ramR*
Fosfomycin			
Fosfomycin	32[c], 16[d]	ND	*fosA*
Polymyxin			
Colistin	>8	NWT	Disrupted *mgrB*
Polymyxin-B	>8	NWT	Disrupted *mgrB*
Sulfonamide			
Trimethoprim-sulfamethoxazole	8/152	R	*sul1*
Tetracycline			
Tetracycline	>32	R	*tet*(A), truncated *ramR*
Tigecycline[b]	4	I	Truncated *ramR*
Macrolide			
Not included in AST panel	Na	Na	*mph*(A)

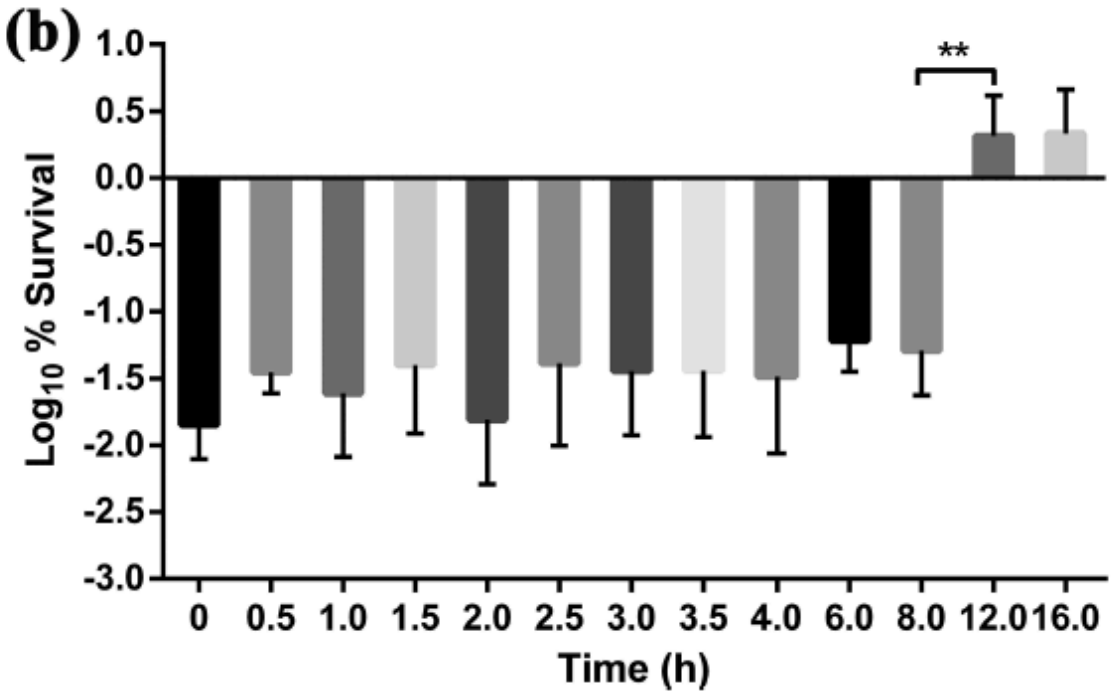
de Man TJB, et al. mBio 2018; 9: e00440-18.

Persister cells are commonly formed with *K. pneumoniae* grown in exponential phase



- Persister cells could play a role in the frequent development of resistance
- Li Y et al. J Medical Microbiol. 2018; 67: 273-281

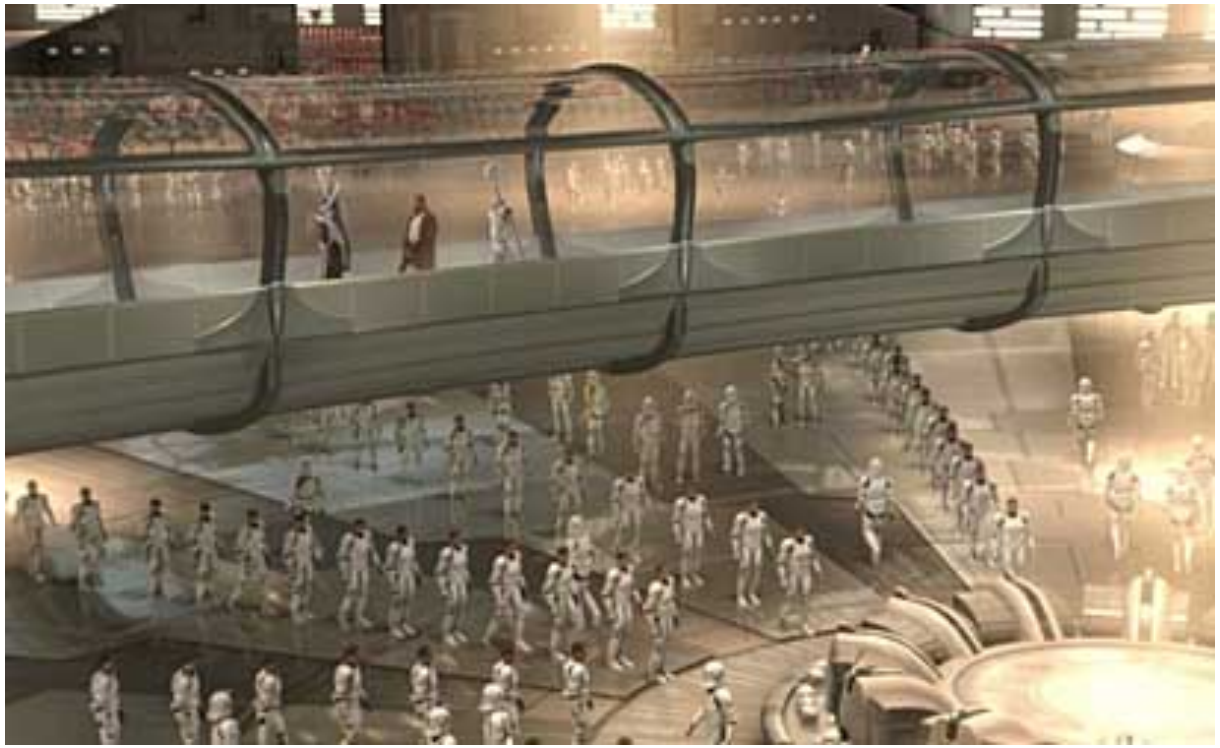
Persister cells in stationary phase can even survive in 100-fold MIC concentrations



- Number of hours from subcultivation
- Statistically significant when comparing late exponential phase and stationary phase (could be of importance in subacute infections/colonization

Bacterial clones

What is a clone?



History of phylogenetic typing

- Multilocus enzyme electrophoresis (MLEE)
  - → Identifies variants of the gene products of 10–20 housekeeping genes (genes encoding basic metabolic functions)
  - → Electrophoresis of cell extracts on starch gels, followed by detection using specific enzyme stains
  - → Problem: interlaboratory variation
- 1998 introduction of multilocus sequence typing (MLST)
  - → Increased resolution, few loci needed
  - → Exportable data and international databases
  - → *N. meningitidis* and *S. pneumoniae* both available in 1998
- Difficult to visualize population structures with trees as the number of sequences increase

Spratt BG. JAC 2012

Clonal expansion – MLST definition (strict)

Isolate	Gene A	Gene B	Gene C	Gene D	Gene E	Gene F	Gene G
1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	2
3	1	1	1	1	1	2	1
4	1	1	1	1	2	2	2

High-Risk Clones (HiRC)

- Associated with resistance of great clinical importance

AND

- High ability to spread in health-care institutions

OR

- High ability to confer invasive disease

OR

- High ability to colonize individuals for long time periods

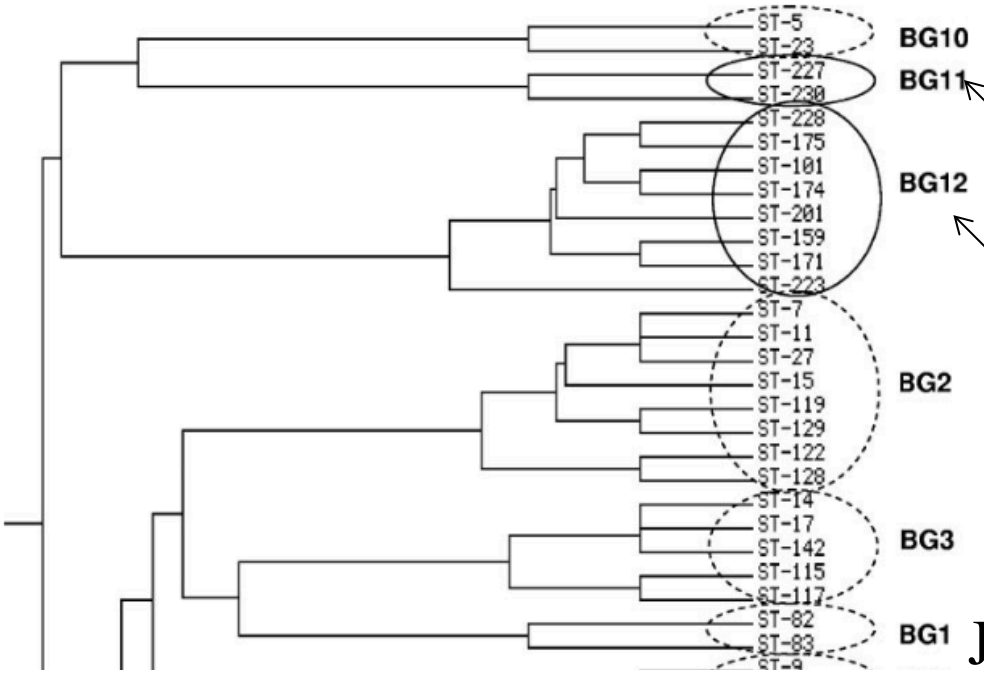
- Perhaps a better term: epidemic clone

Some early work on epidemic clones

Karolinska Institutet

Establishing Clonal Relationships between VIM-1-Like Metallo-β-Lactamase-Producing *Pseudomonas aeruginosa* Strains from Four European Countries by Multilocus Sequence Typing▽

Christian G. Giske,[1]†* Balázs Libisch,[2]†* Céline Colinon,[3]‡ Effie Scoulica,[4] Laura Pagani,[5] Miklós Füzi,[2] Göran Kronvall,[1] and Gian Maria Rossolini[3]



Later CC235

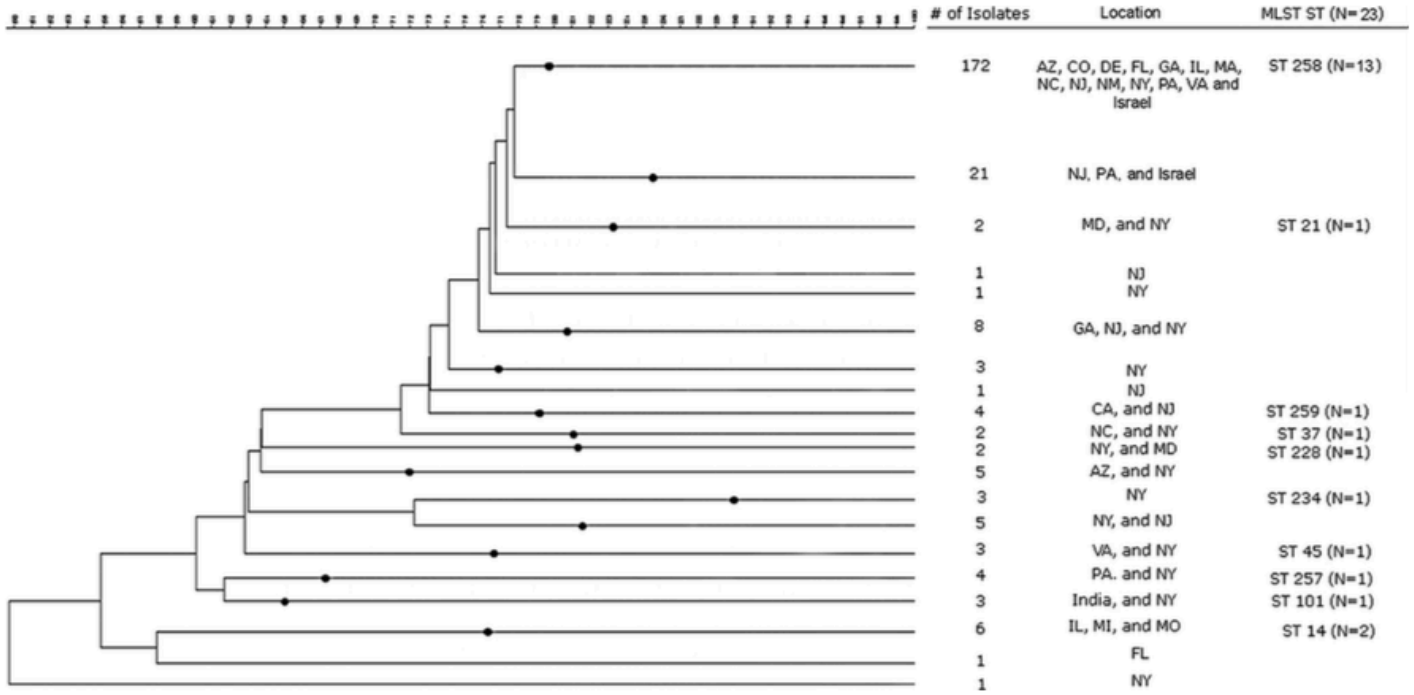
Later CC111

JCM 2006; 12: 4309

Early work in KPC-producing *K. pneumoniae*

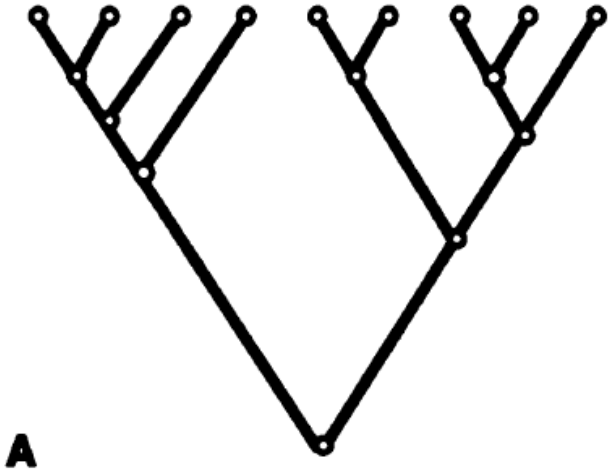
Molecular Epidemiology of KPC-Producing *Klebsiella pneumoniae* Isolates in the United States: Clonal Expansion of Multilocus Sequence Type 258[▽] AAC 2009; 8: 3365

Brandon Kitchel,[1]* J. Kamile Rasheed,[1] Jean B. Patel,[1] Arjun Srinivasan,[1] Shiri Navon-Venezia,[2] Yehuda Carmeli,[2] Alma Brolund,[3] and Christian G. Giske[3]

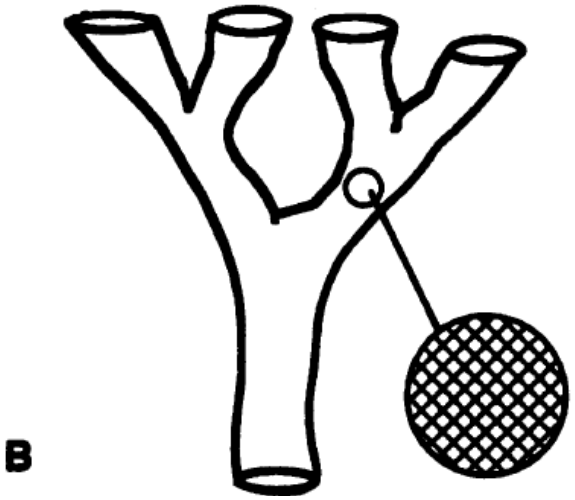


PFGE-database: n=248

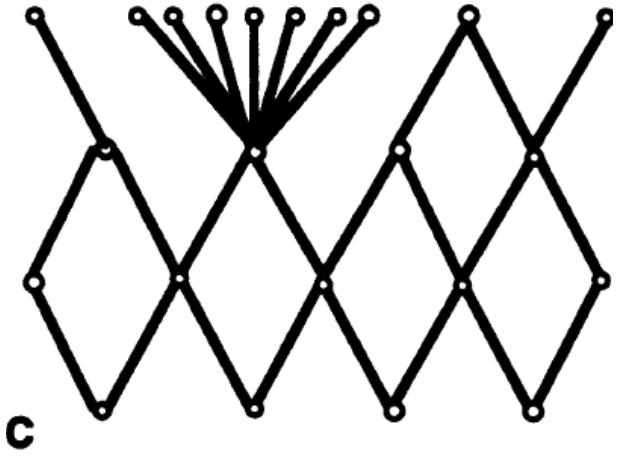
Population structures



Clonal, no recombination



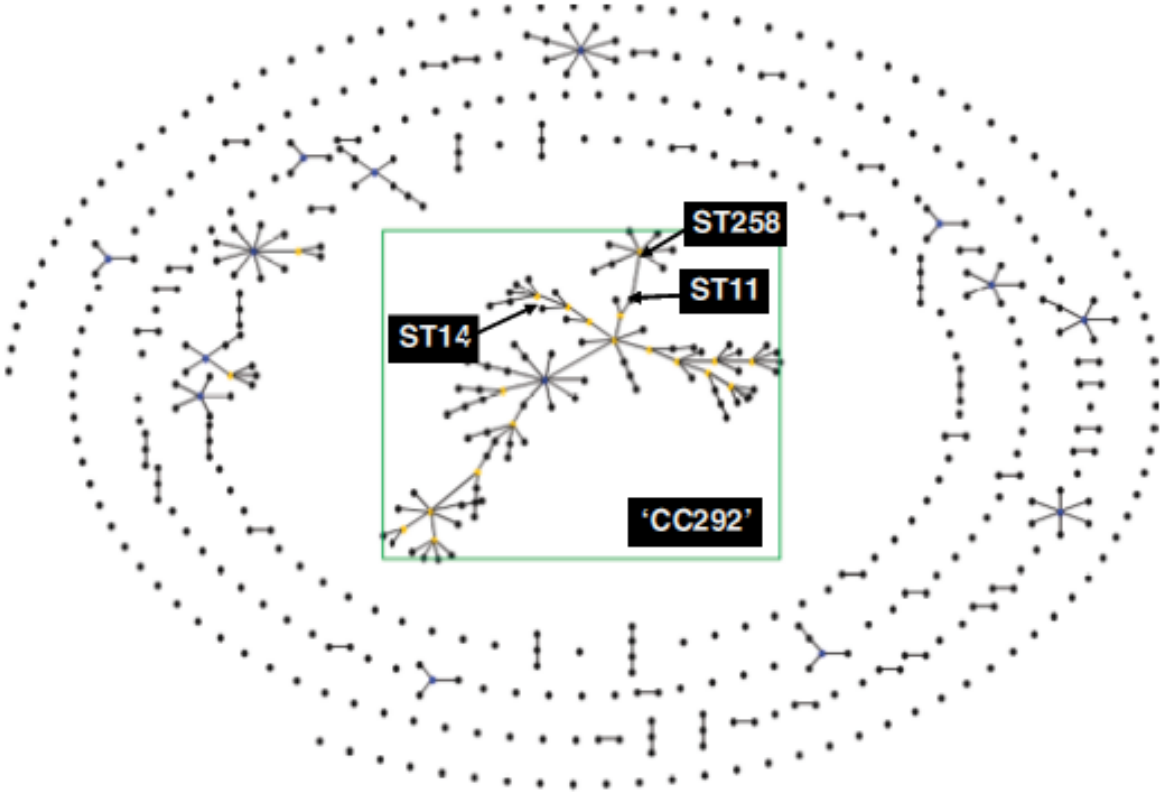
Recombination within main branches



Panmictic/epidemic

Smith JM. PNAS 1993

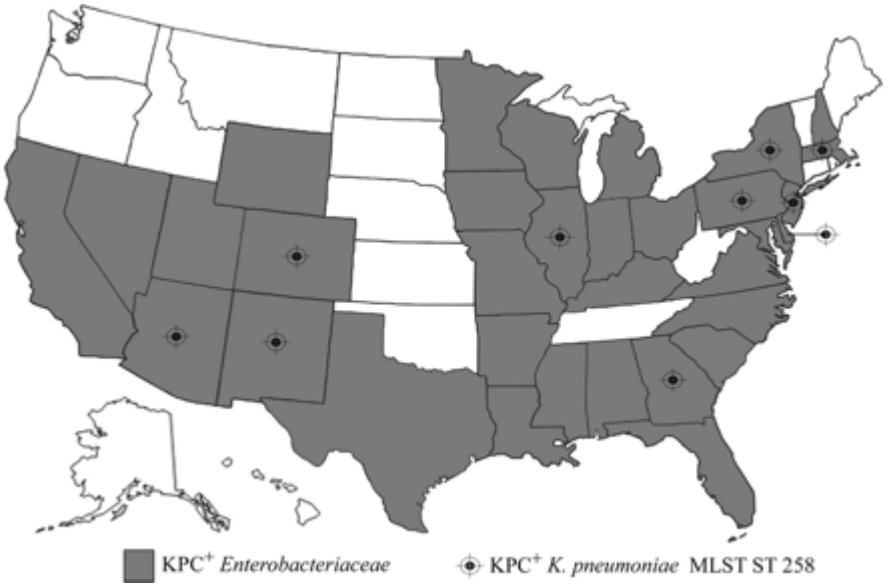
Poplation snapshot *K. pneumoniae*



Woodford et al. FEMS Microbiol Lett 2011

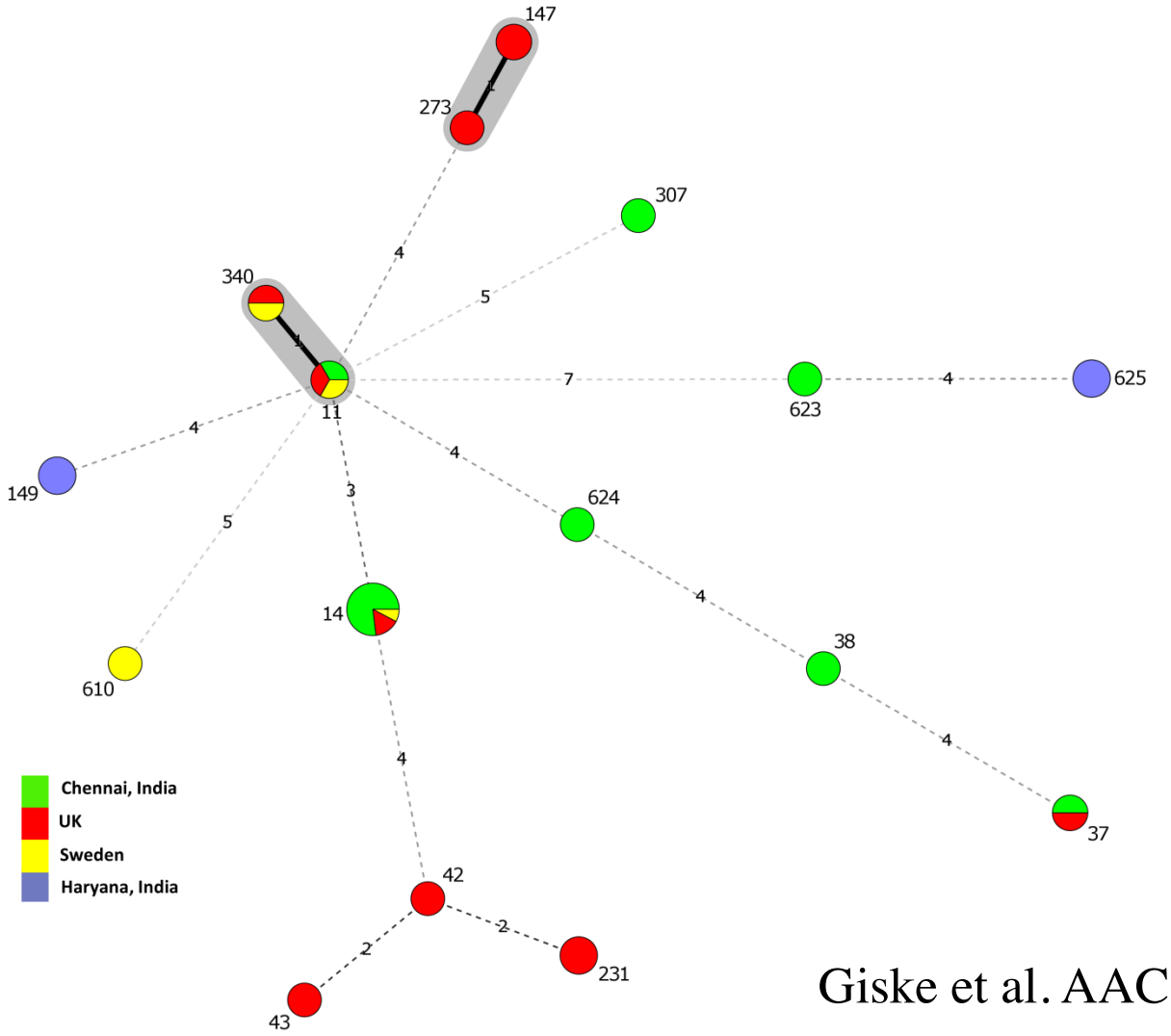
K. pneumoniae ST258

- Clone associated with the spread of the carbapenemase KPC
- Initially described in USA, then in most countries where KPC has been detected
- Responsible for 70% of the cases of KPC in the US and most cases in Israel
- Also capable of causing invasive disease



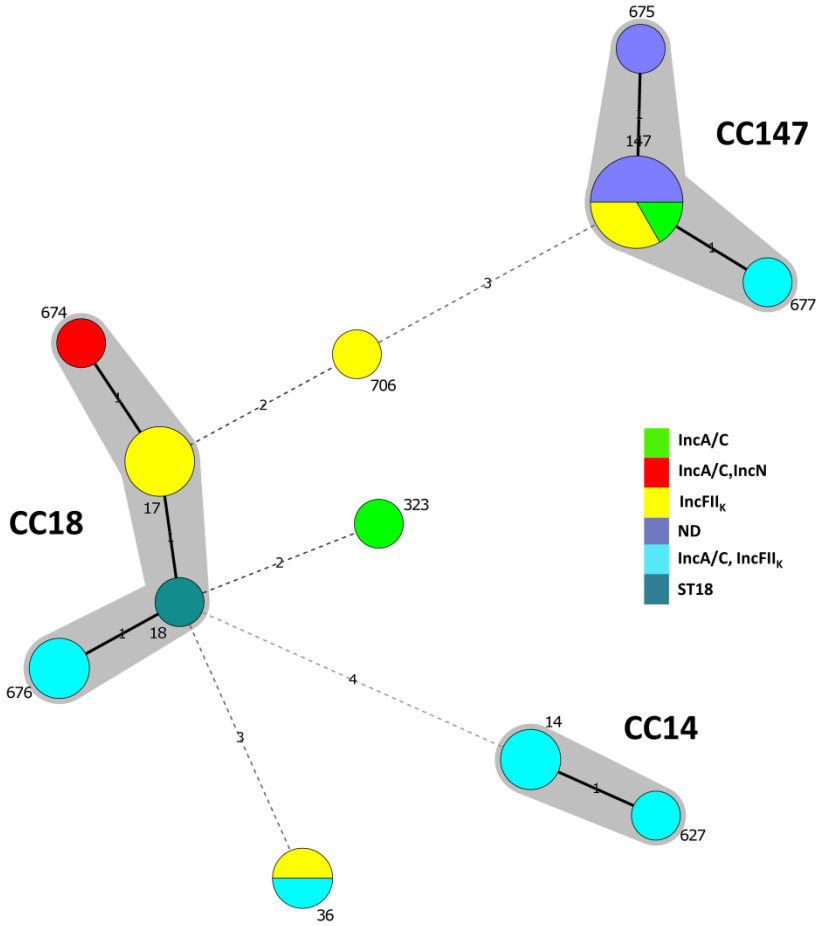
Kitchel et al. AAC 2009

Sequence types in NDM-produces



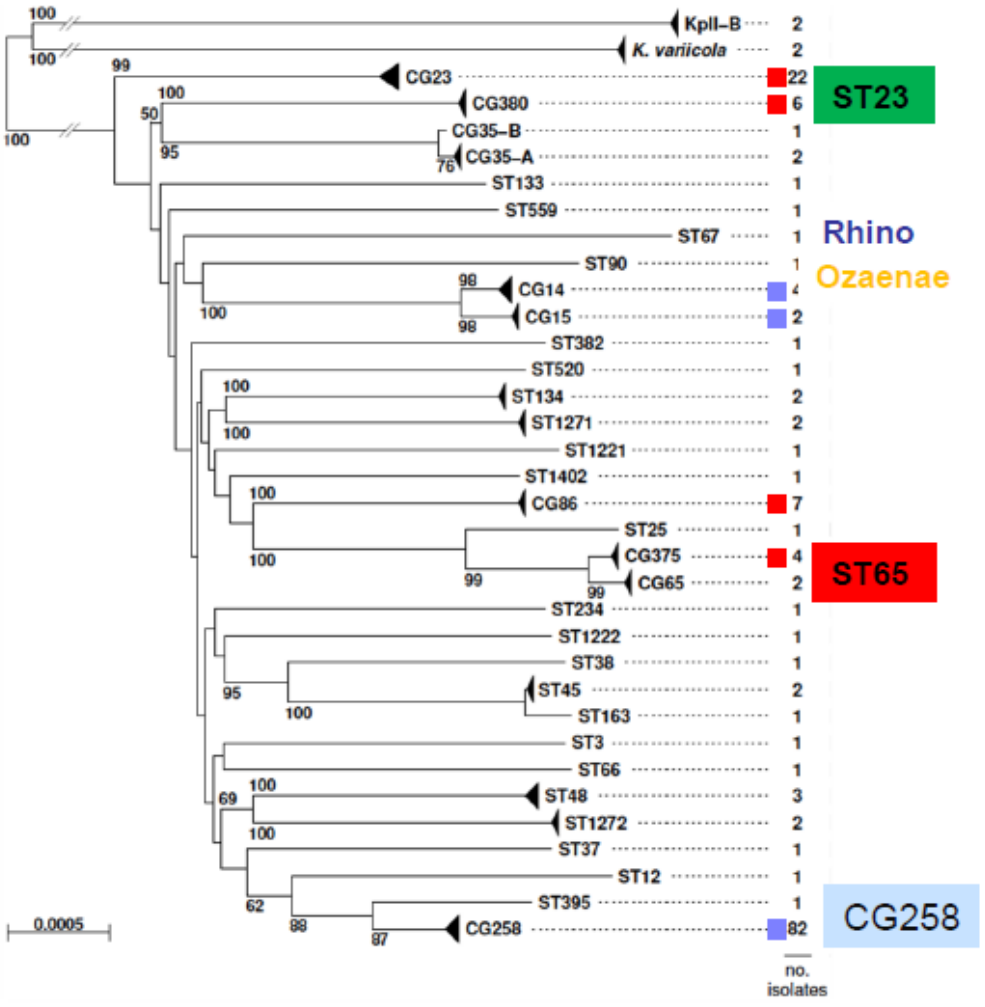
Giske et al. AAC 2012

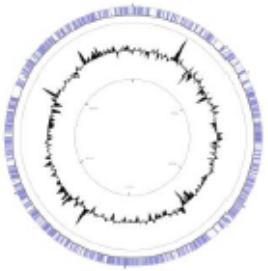
Sequence type in VIM-producers



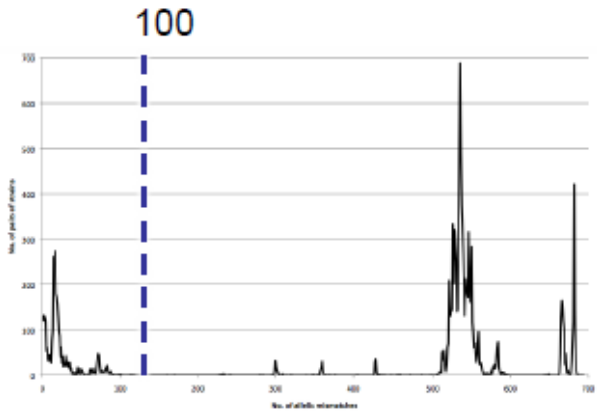
Hasan et al. CMI 2013

Correlation cgMLST and MLST





694 core genes

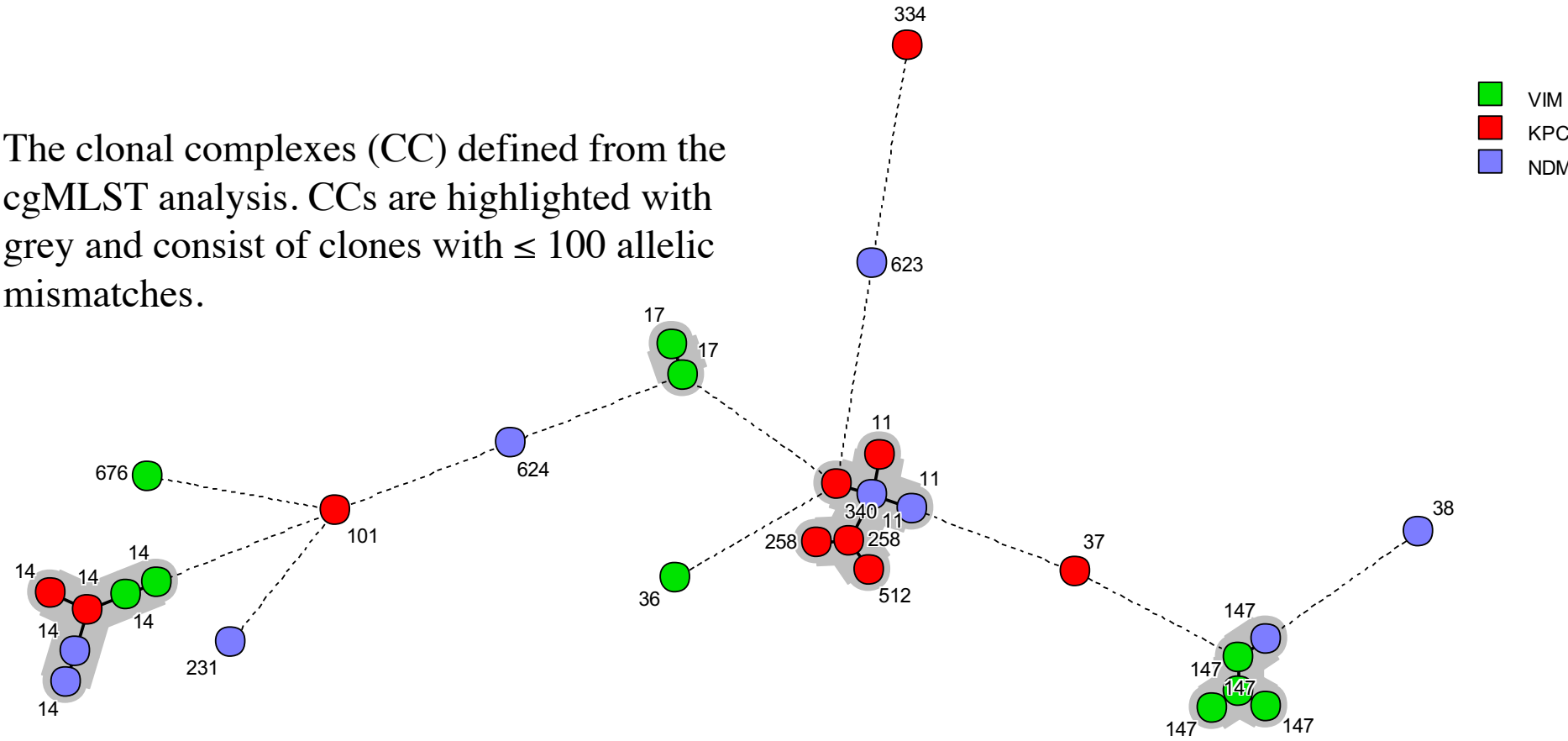


No. Allelic mismatches

Epidemic clones of *K. pneumoniae*

Overview of strain collection

The clonal complexes (CC) defined from the cgMLST analysis. CCs are highlighted with grey and consist of clones with ≤ 100 allelic mismatches.



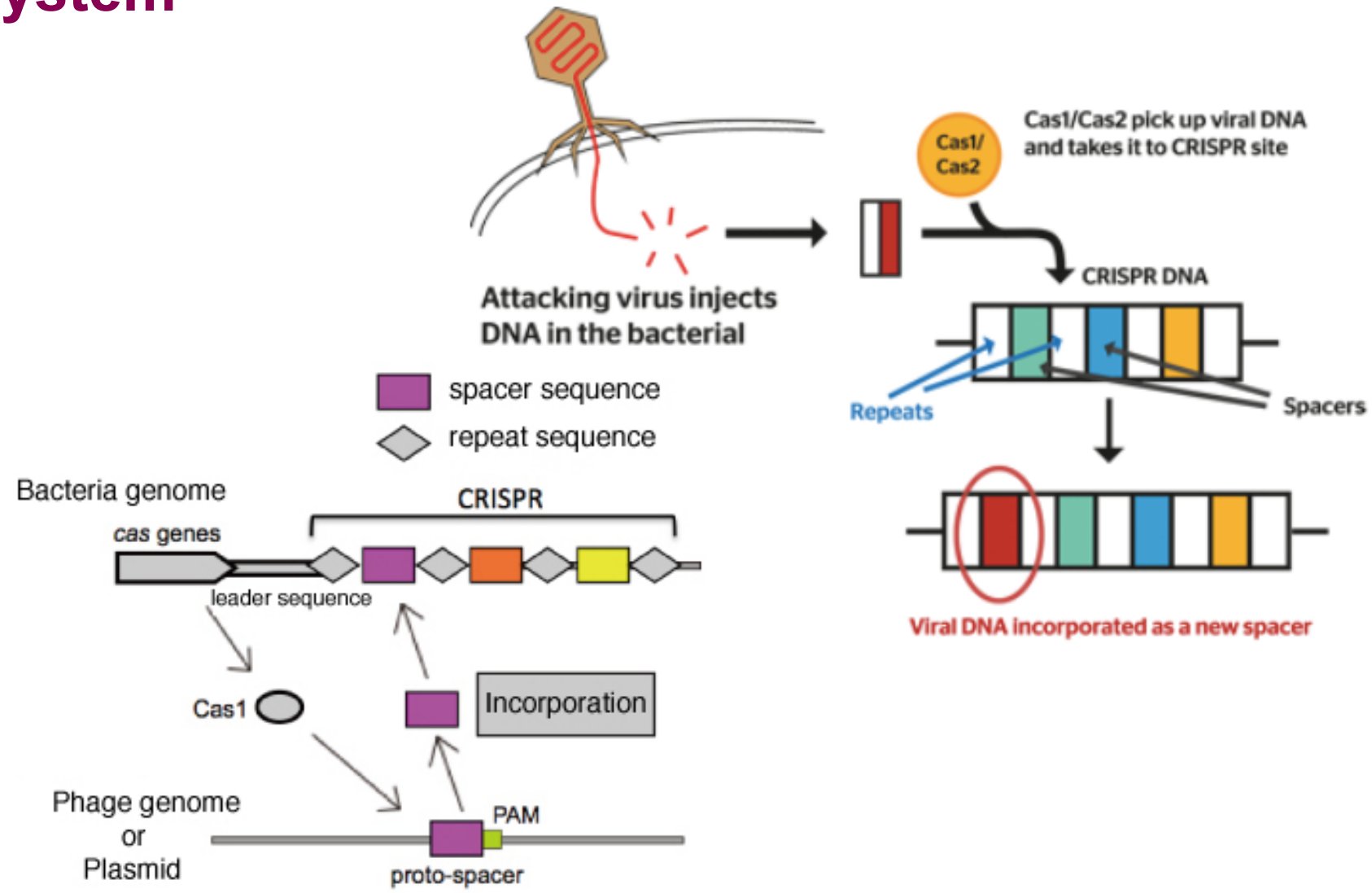
Örmälä-Odegrip A et al. 2017. Manuscript

Searching for differences between epidemic and non-epidemic clones in the genome

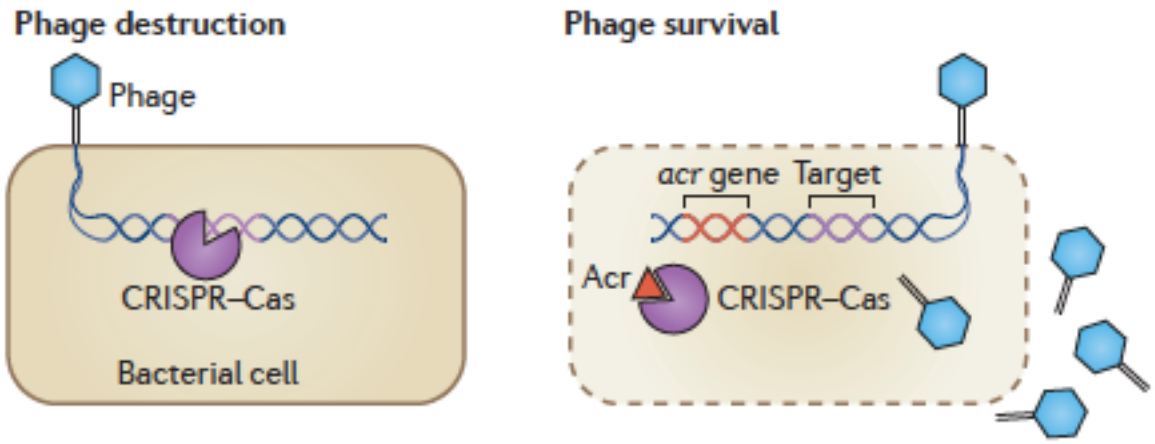
Feature	Difference epidemic vs non-epidemic
Virulence genes	Few virulence genes in both groups
Prophages	No quantitative difference between groups
CRISPR-Cas	Higher occurrence in epidemic ($p<0.01$)

Örmälä-Odegrip A et al. Manuscript

CRISPR-Cas: the bacterial immune system



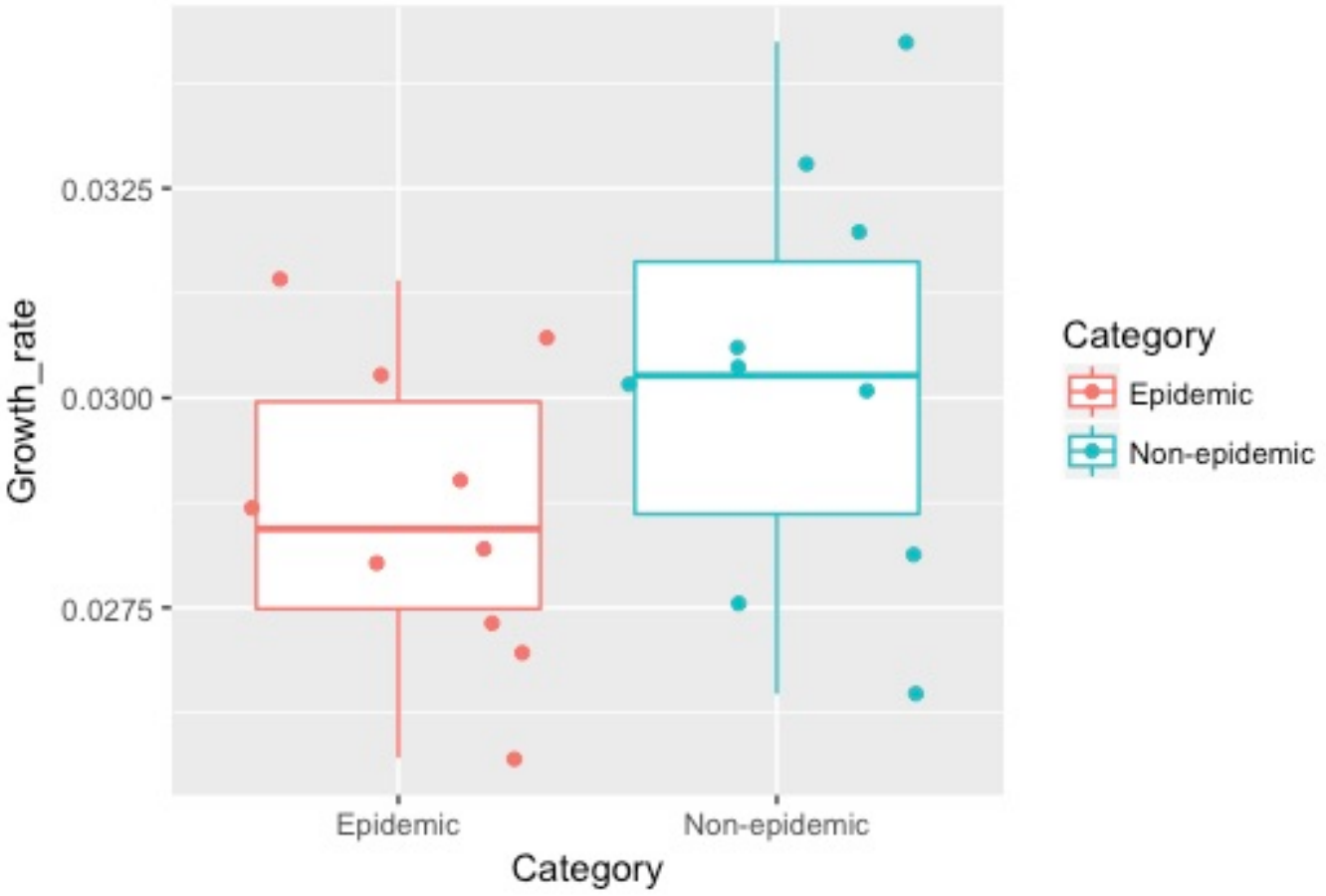
Anti-CRISPR: phages and mobile elements evade CRISPR-Cas immunity



Pawluk A. Nat Rev Micro 2017

Growth rate (Bioscreen)

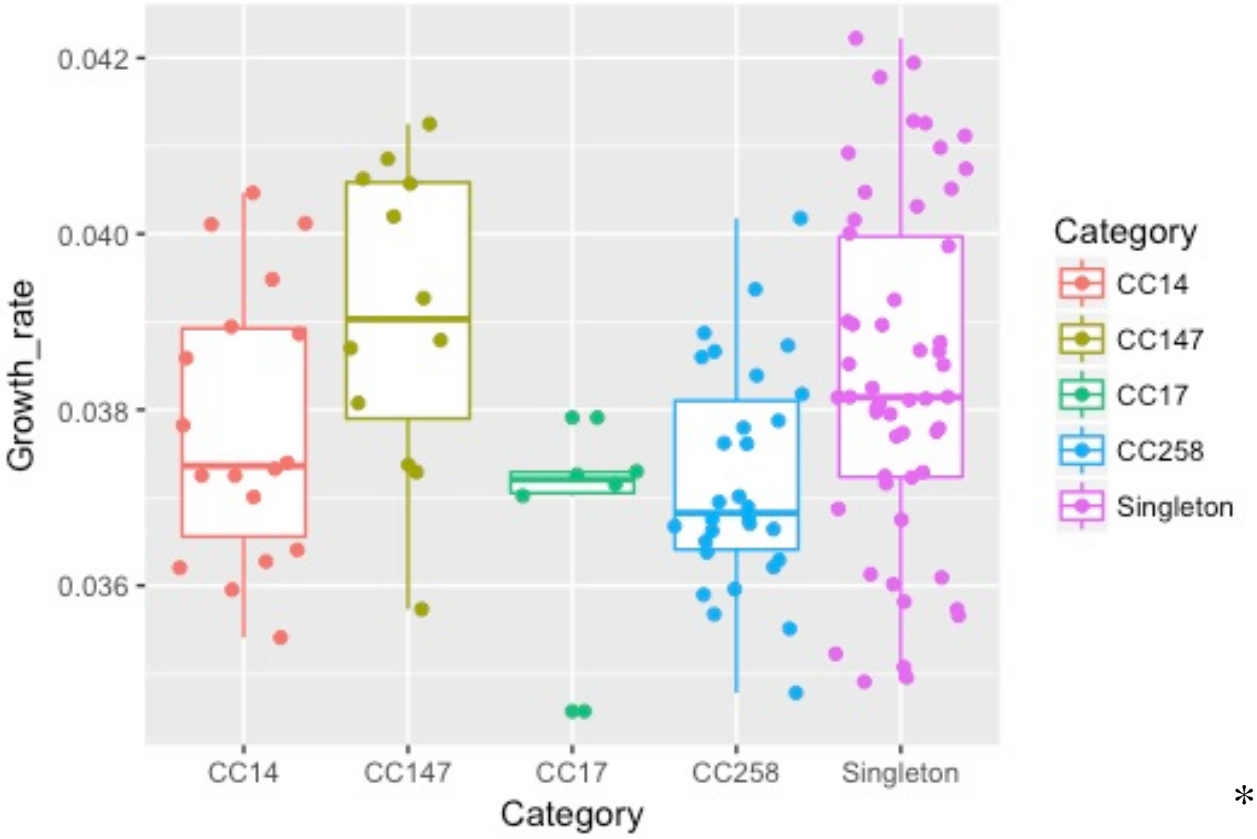
Growth rate, bioscreen



GLM (ANOVA)
F =8.406
df= 1
Sig.= 0.05 level

Student T-test
Sig.= 0.005 level
Non-epidemic isolates grow faster

Growth rate per CC



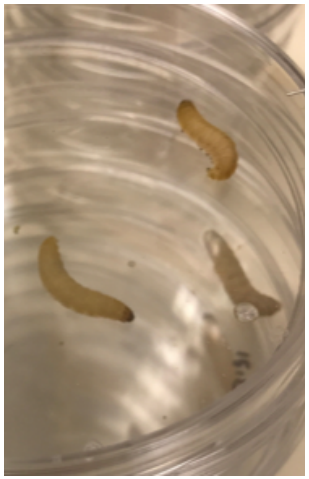
GLM (ANOVA)
F =4.326
df= 4
Sig.= 0.003

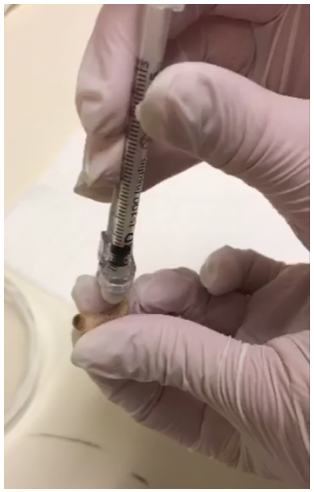
Post Hoc *
CC258 < CC147, Singleton
CC147 > CC258, CC17
Singleton > CC258, CC17

* The mean difference is significant at 0.05 level

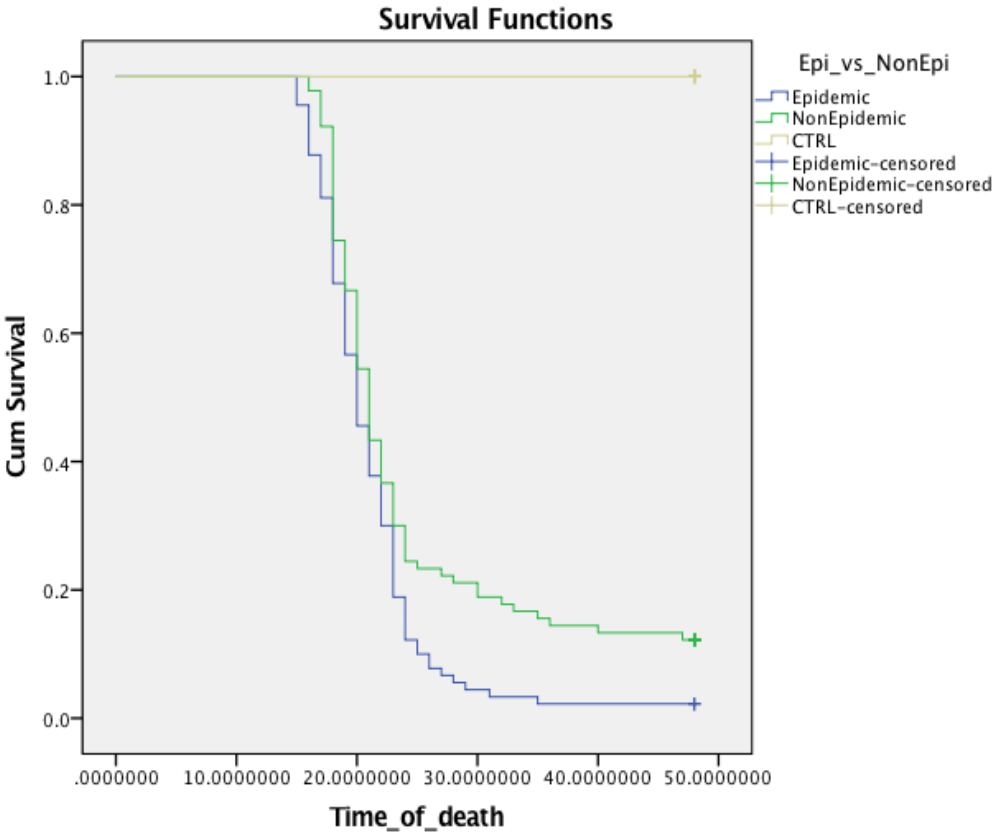
Virulence (*Galleria mellonella*)

Galleria mellonella



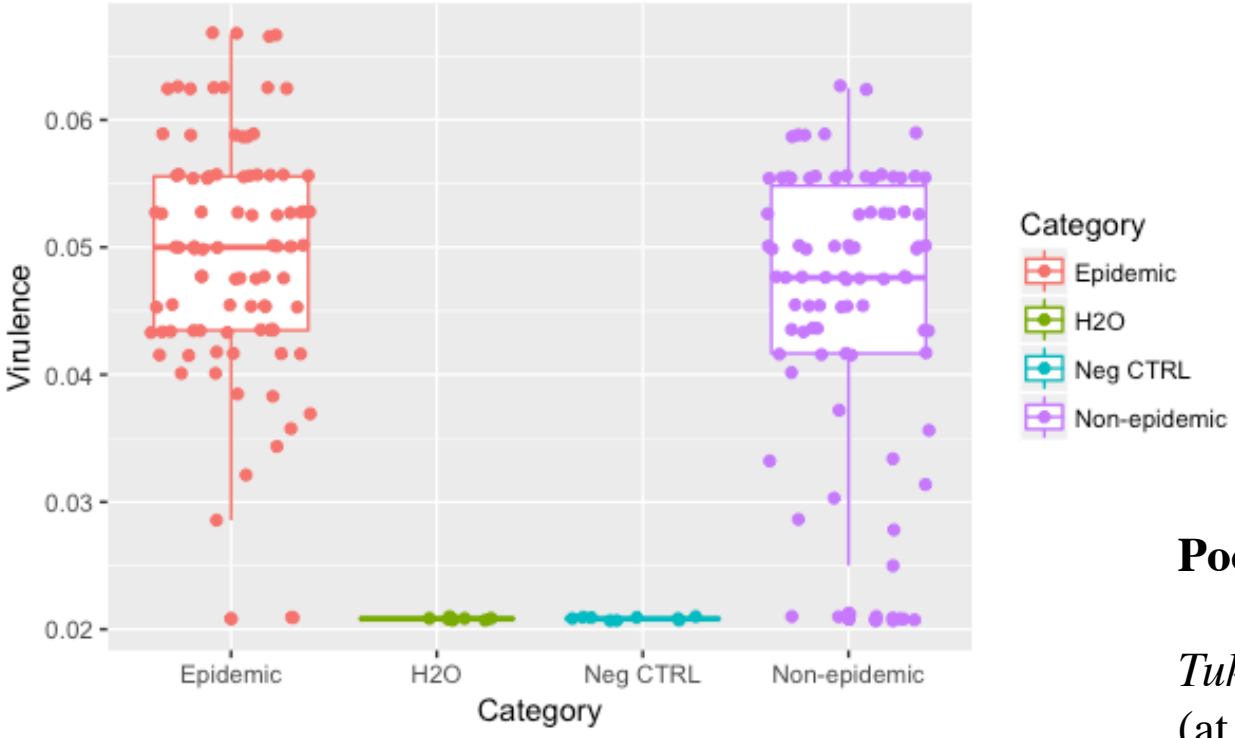



Survival epidemic vs non-epidemic



Overall Comparisons			
	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	7.536	1	.006
Breslow (Generalized Wilcoxon)	4.291	1	.038

Virulence: epidemic>non-epidemic



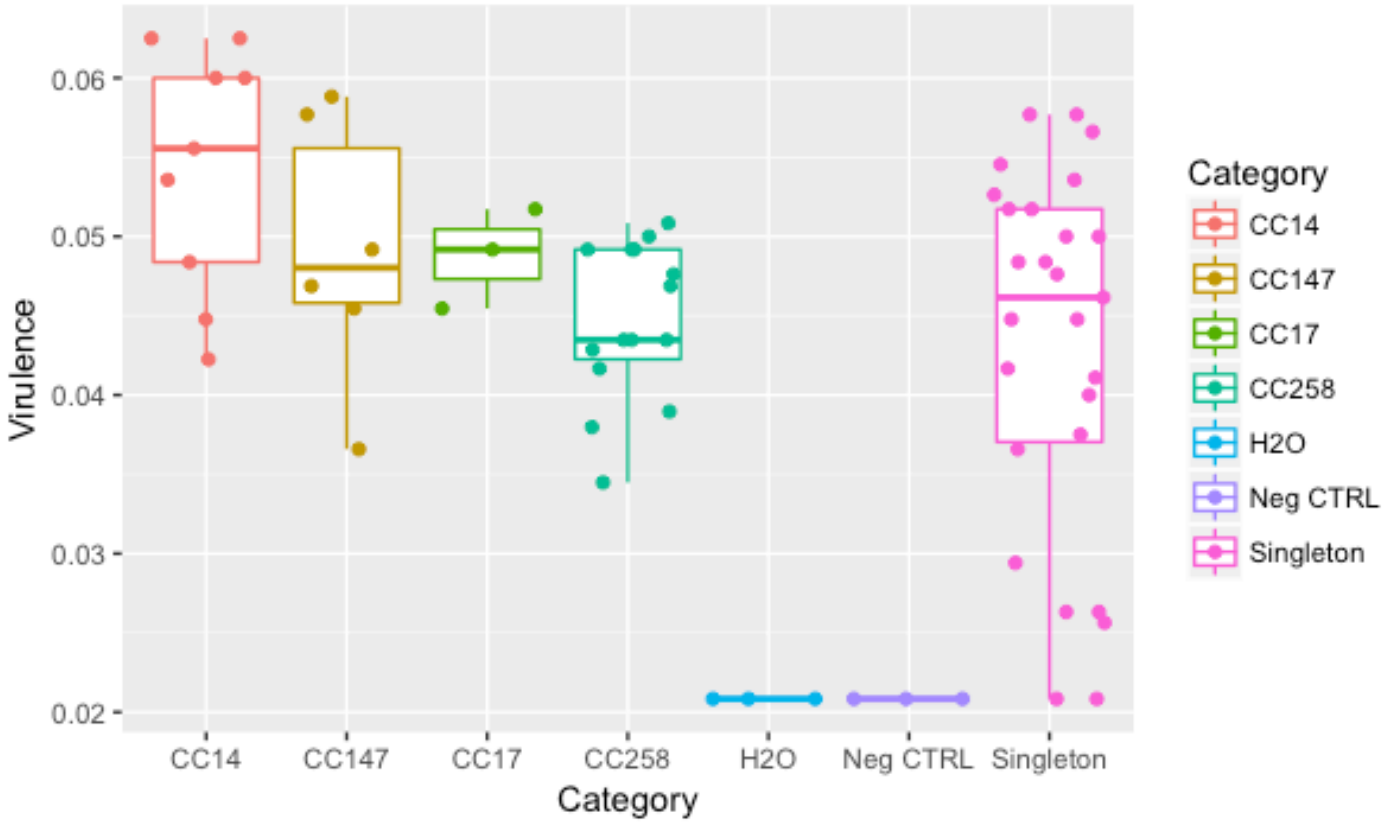
GLM (Epi vs Non-epi)
df= 1
F= 9.099
Sign.= 0.003

Poct Hoc: multiple comparison

Tukey: Epidemic > Non-epidemic (at sig 0.05)
LSD: Epidemic > Non-epidemic (at sig 0.02)

Mean difference is significant

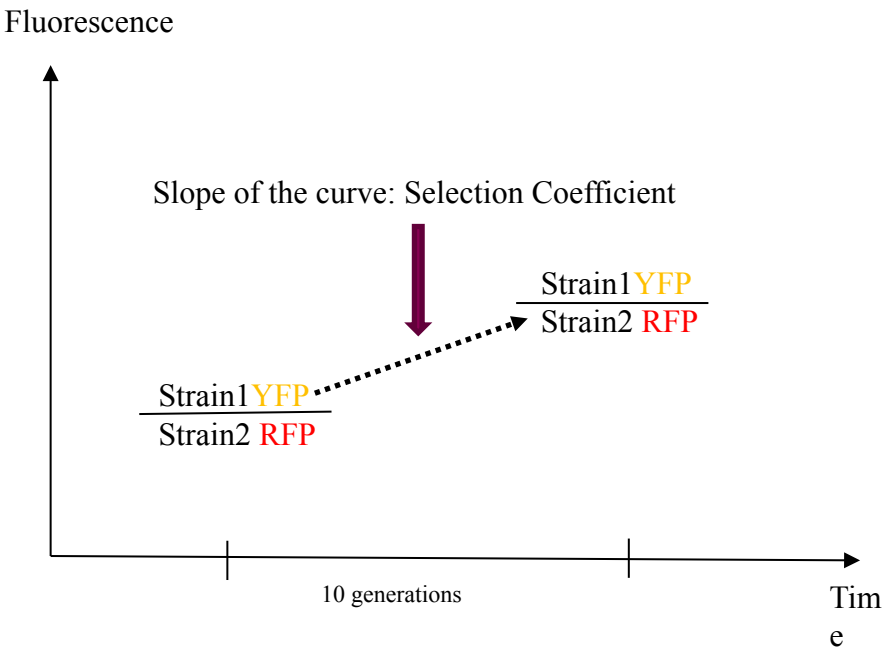
Virulence vs clonal complex



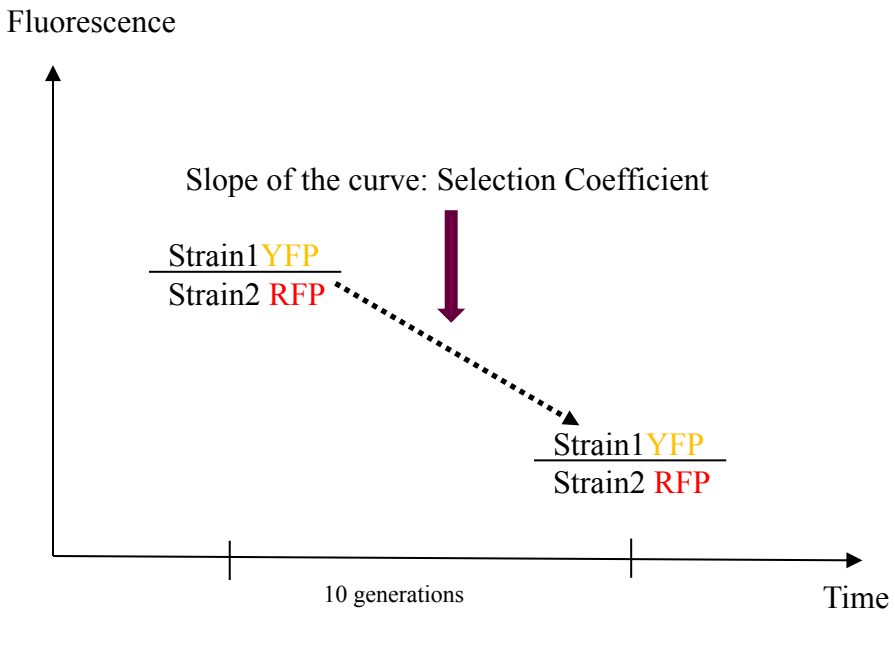
GLM (Clonal Complex)
df= 4
F= 2.876
Sign.= .031

Liquid competitions (MACS)

Competition experiments: magnetic-activated cell sorting (MACS)



Positive slope→ (+) S-value: Strain 1 wins over Strain 2

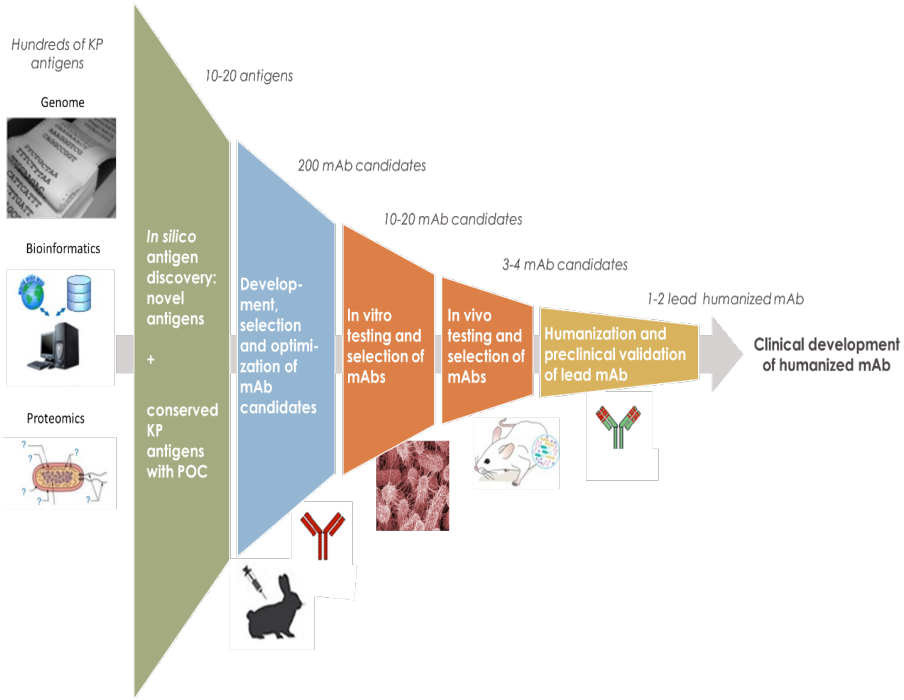


Negative slope→ (-) S-value: Strain 1 looses against Strain 2

Preliminary assessment: non-epidemic strains seem to win over epidemic strains in majority of cases

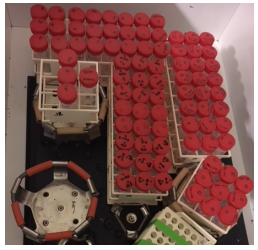
Novel treatment concepts

Novel treatment concepts

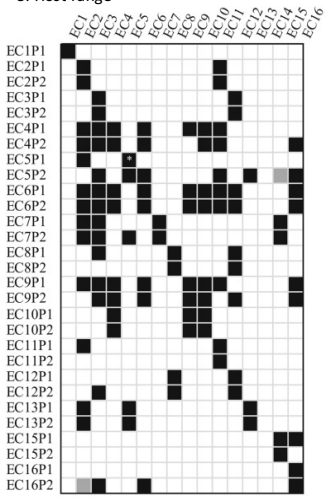


1. Sample collection

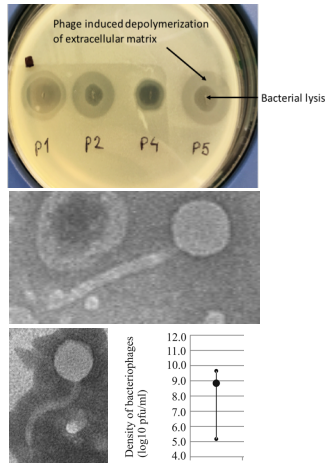
2. Phage enrichment / isolation



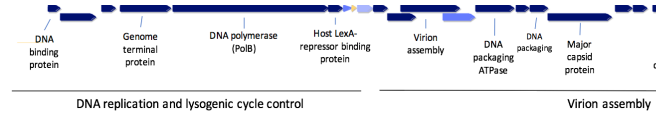
3. Host range



4. Phenotypic characterization



5. Genomic analysis



Conclusions

- In some regions *K. pneumoniae* infections are observed in multimorbid individuals
- Successful (or epidemic) clones are responsible for a major share of dissemination of antimicrobial resistance
- Noteworthy among resistance mechanisms are particularly the carbapenemases
- Heteroresistance may be important in resistance to several drug classes – possible to suppress with combination therapy?
- Molecular typing tools have helped us define important clones
- The factors determining why some clones are epidemic are still largely unknown – detecting them only a first step
- Novel treatment concepts: phages and antibodies