

The complex and diverse epidemiologies of ESBLs and carbapenemases



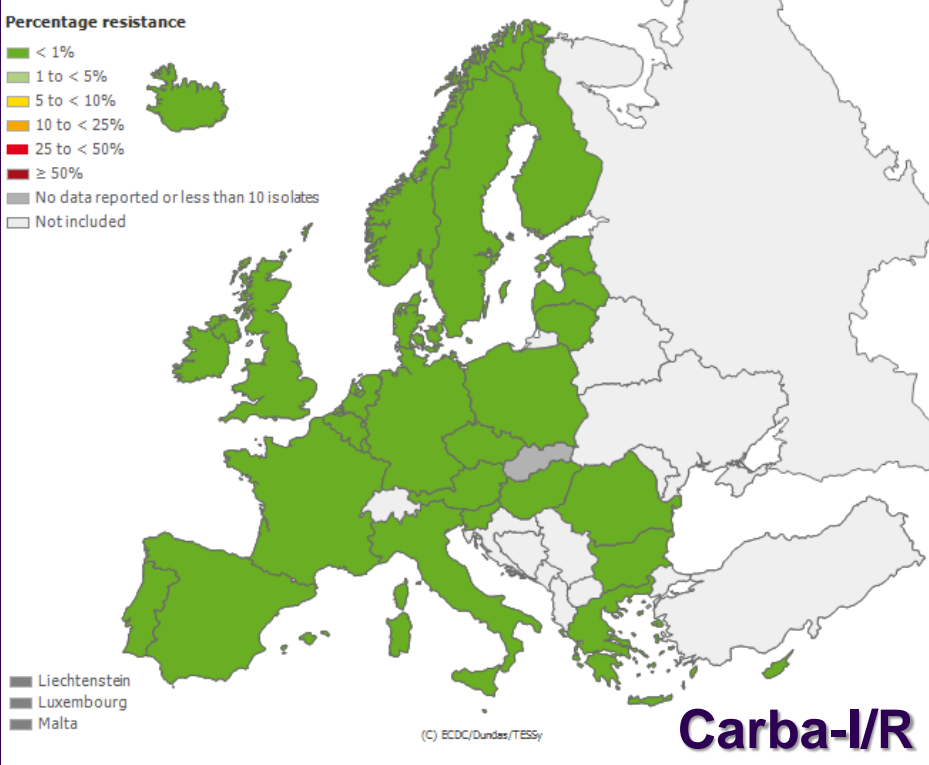
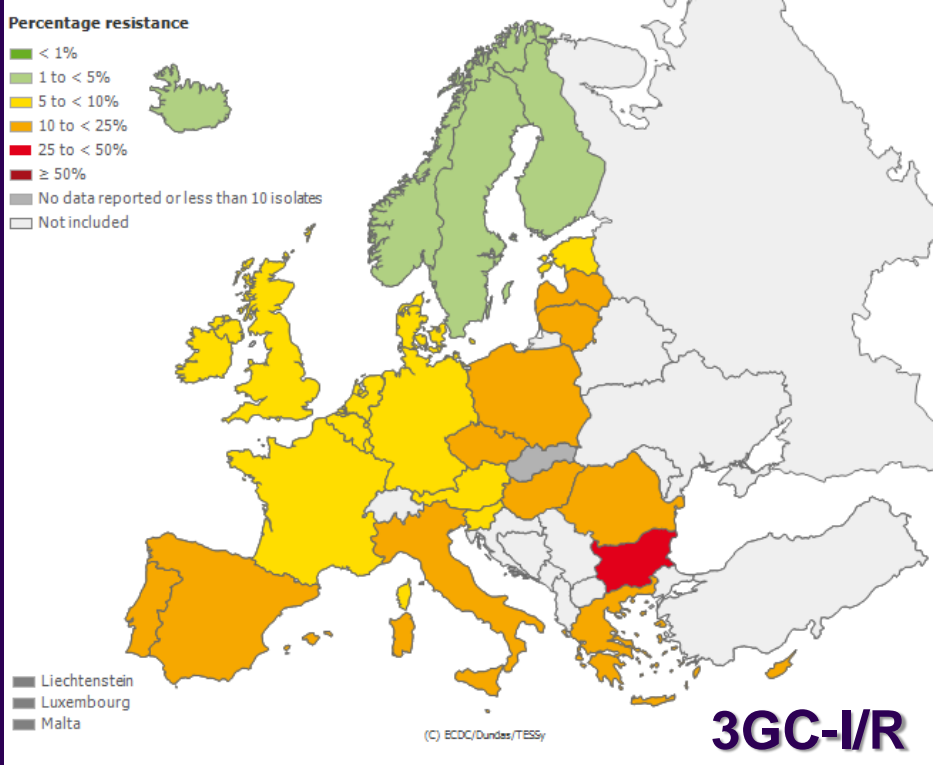
Neil Woodford

HPA – ARMRL - Colindale



ESCMID
COLLABORATIVE CENTRE

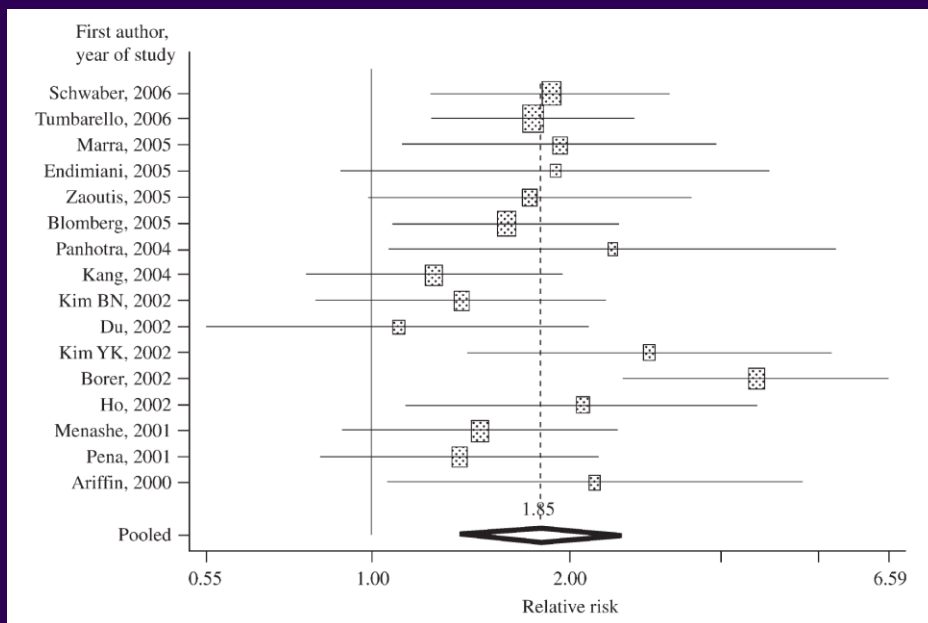
E. coli, 2010 (Ears-Net)



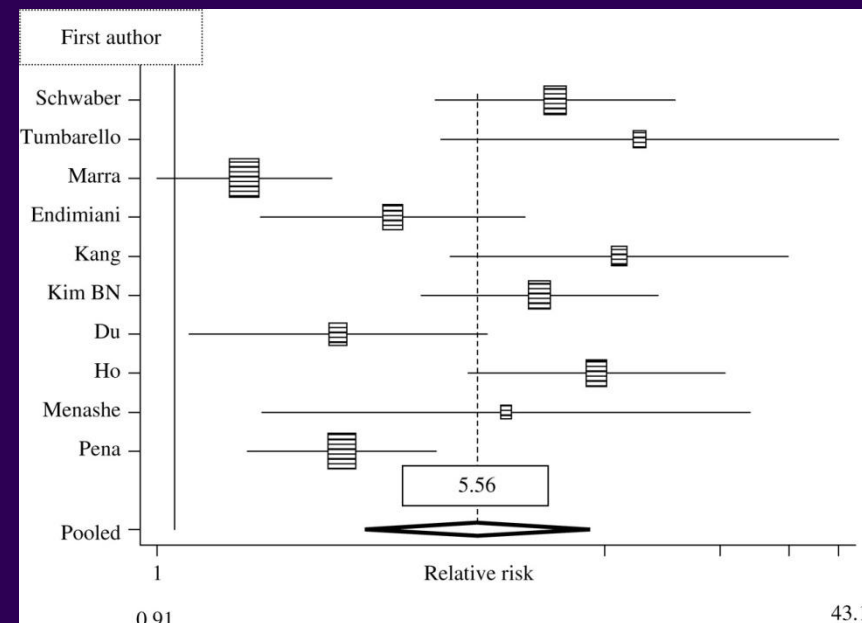
- In the UK:

- c. 30,000 cases *E. coli* bacteraemia p.a.
- c. 6.5 % CTX and/or CAZ resistance = c. 2000 cases p.a

ESBL vs. non-ESBL bacteraemia

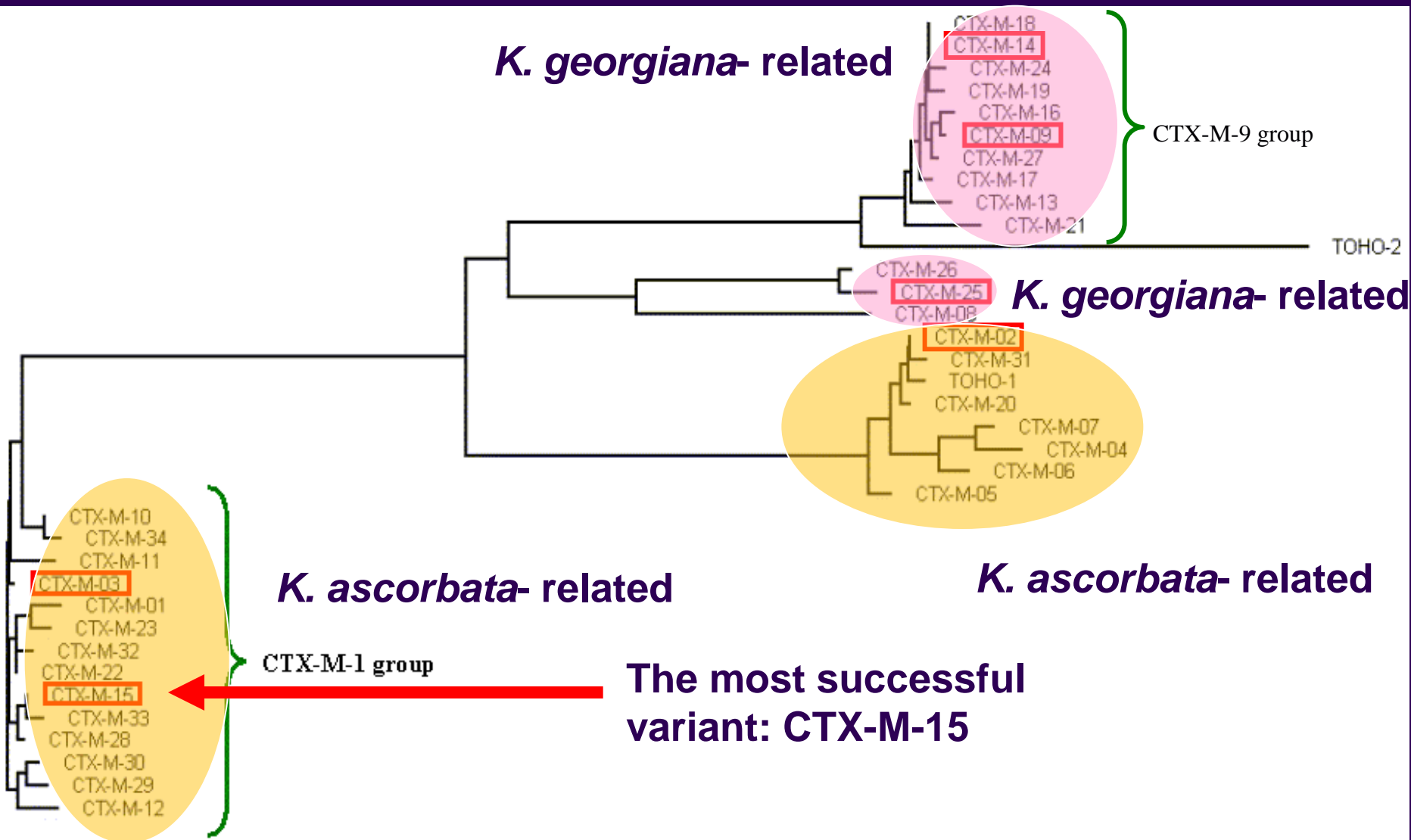


Mortality



Delayed appropriate Rx

A multitude of CTX-M ESBLs



- ~1500 CTX-M-producing *E. coli* analysed
 - 91% contain alleles encoding group 1 enzymes (mainly CTX-M-15)
 - massive clonal spread
 - 8.5% contain alleles encoding group 9 enzymes
 - diverse strains, some intra-centre strain spread
 - 8 isolates contain an allele encoding group 2 enzymes
 - 1 isolate contains an allele encoding a group 8 enzyme

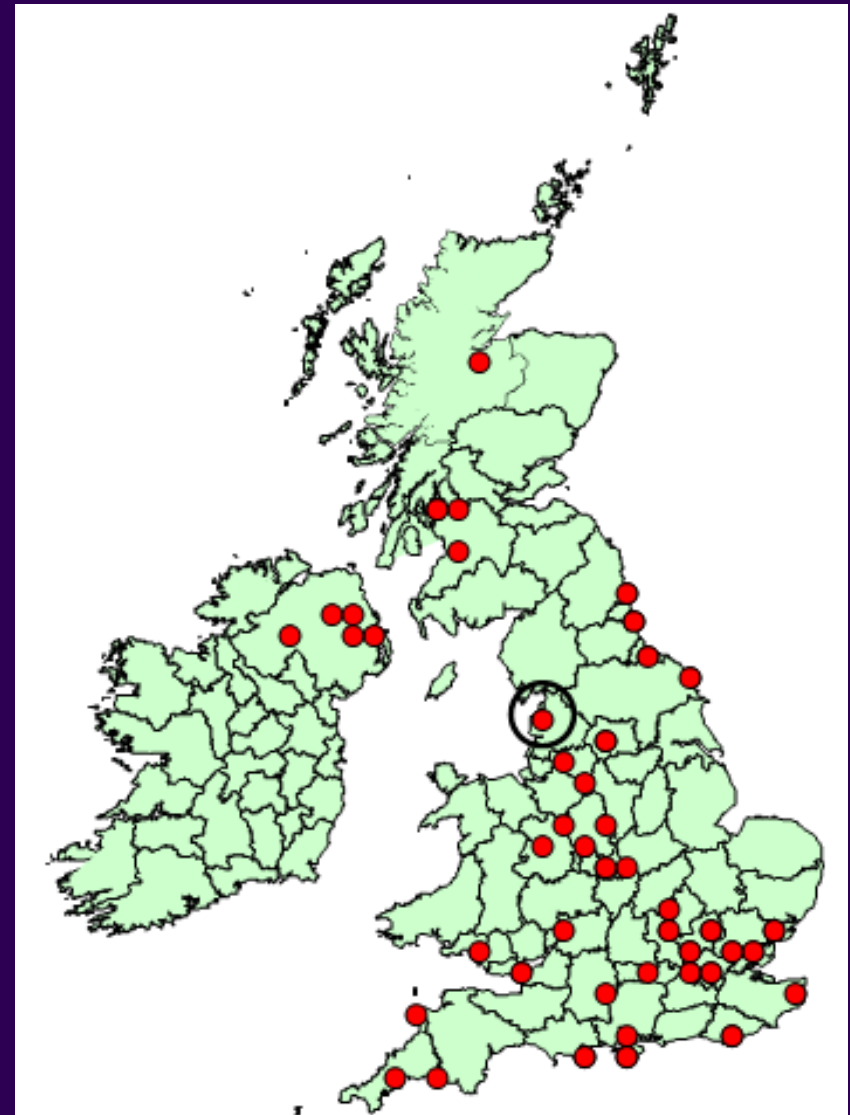
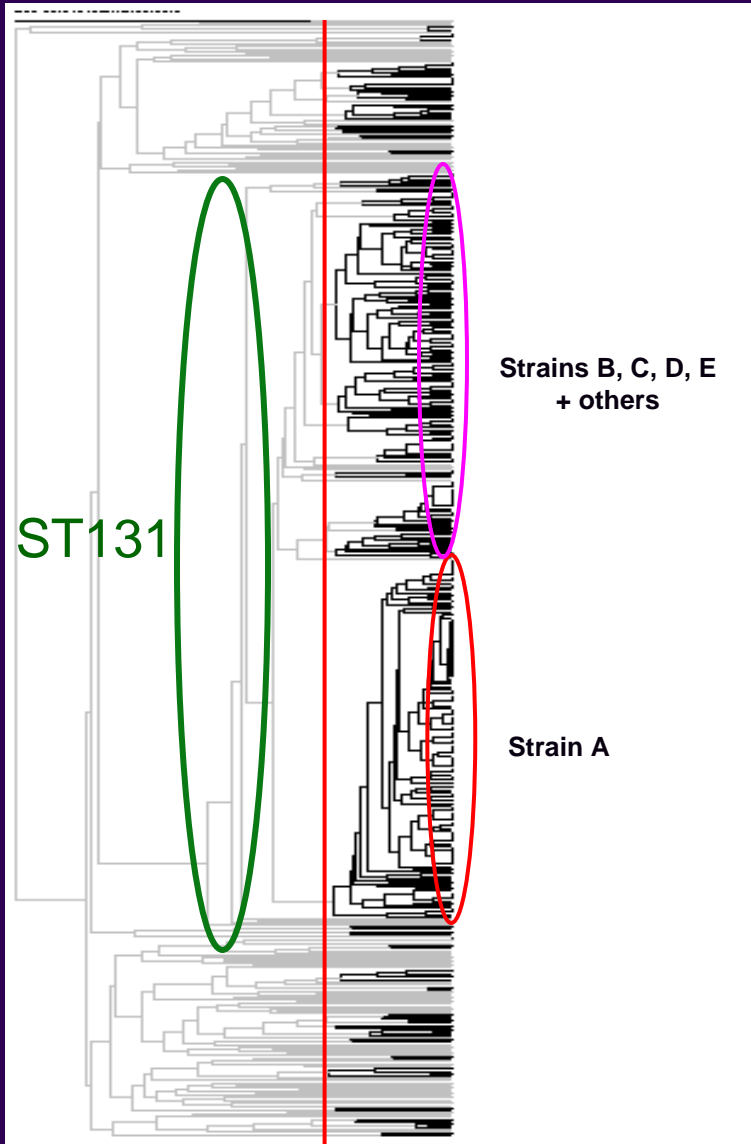
Which *E. coli* clones cause UTI?



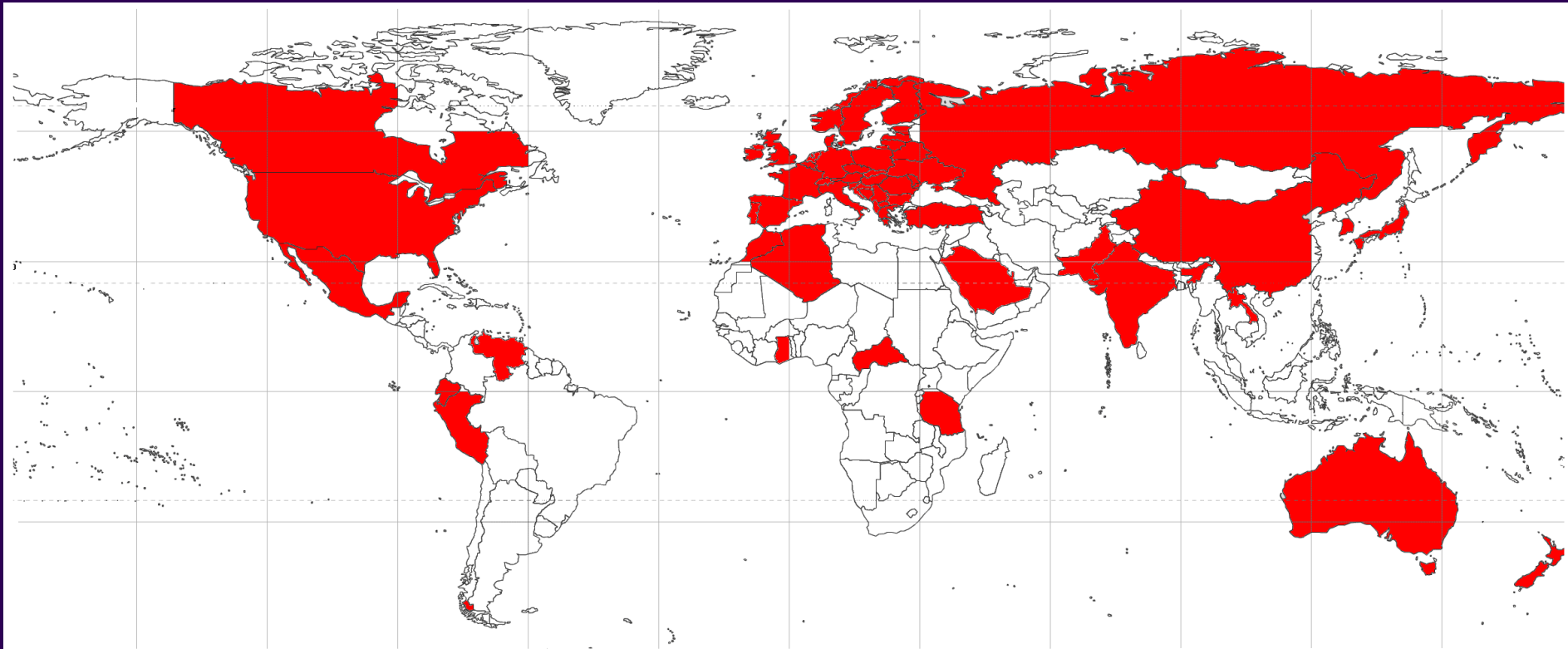
ST		UTIs (n=300)*
73		16.6%
95		6.3%
131		12.3%
12		0.7%
127		3.6%
69		9.0%
Other		51.5% (97 STs)

* Gibreel *et al.* JAC 2012; 67: 346-56

ST131 and spread of CTX-M-15-producing *E. coli* in the UK



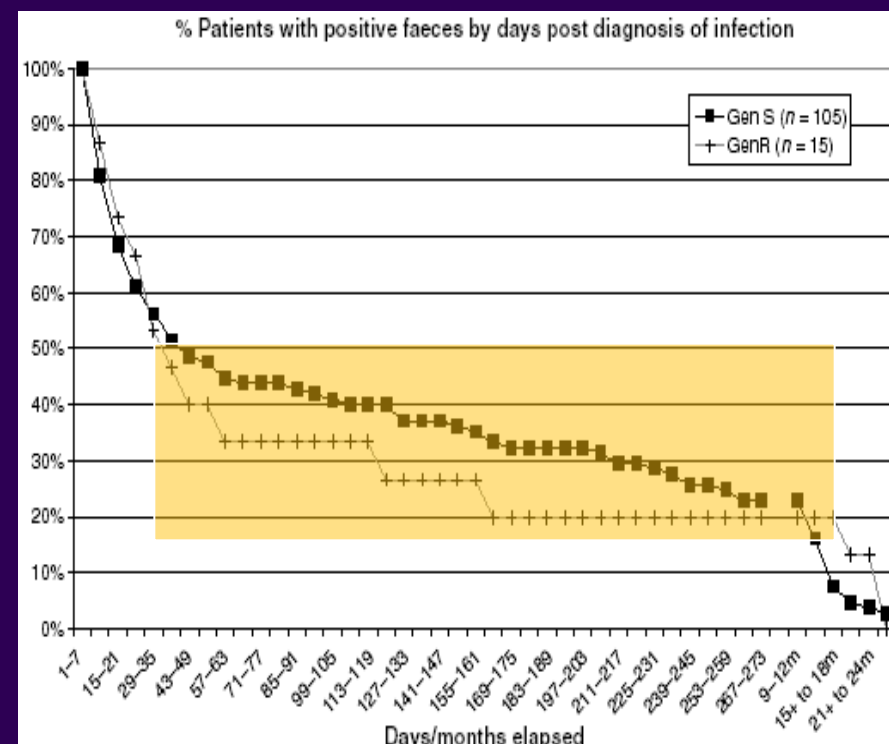
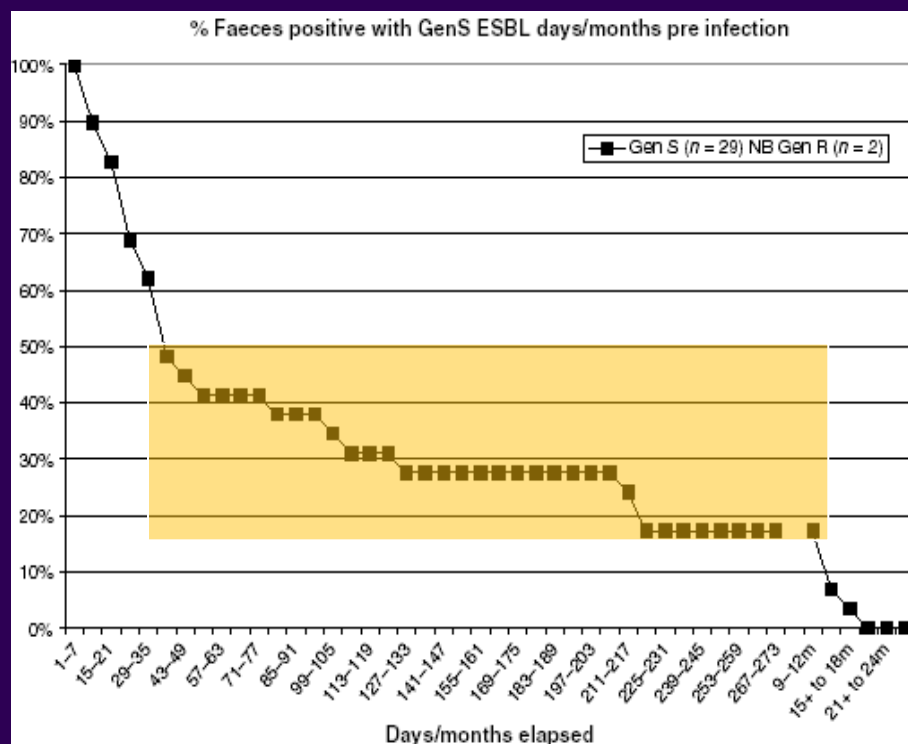
O25-H4:ST131 *E. coli*: a global clone



PFGE sub-divides the
lineage and
allows local
epidemiological
investigations

Nicholas-Chanoine *et al.* *JAC* 2008, **61**, 273
Coque *et al.* *EID* 2008 **14**, 195
Lau *et al.* *JAC* 2008; **62**: 1241-4

Prolonged faecal carriage of ST131 variants: UK strains A & D

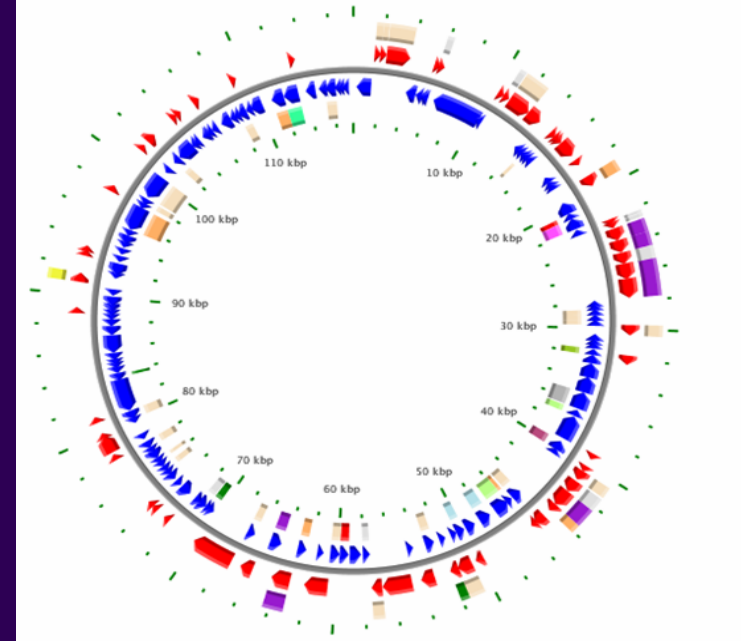


- Potential for persistence in community settings
- 50% colonized for up to 1 month
- 30% colonized between 1 - 12 months

Multi-resistance plasmids help to spread CTX-M -15 ESBL



Antibiotic classes	Genes	Mechanism
Aminoglycosides	<i>aac6'-Ib-cr</i> <i>aadA5</i>	Modify drug
β -lactams	<i>bla</i> _{CTX-M-15} <i>bla</i> _{OXA-1} <i>bla</i> _{TEM-1}	Destroy drug
Chloramphenicol	<i>catB4</i>	Modify drug
Macrolides	<i>mph(A)</i>	Efflux
Fluoroquinolones	<i>aac6'-Ib-cr</i>	Modify drug
Sulfonamides	<i>sulI</i>	By-pass
Trimethoprim	<i>dhfr</i> _{XVII}	By-pass
Tetracycline	<i>tet(A)</i>	Efflux



Thursday September 28 22:33:05 2006 Length: 117,536 bp; Genes: 185

The biogeography / ecology of resistance is complex

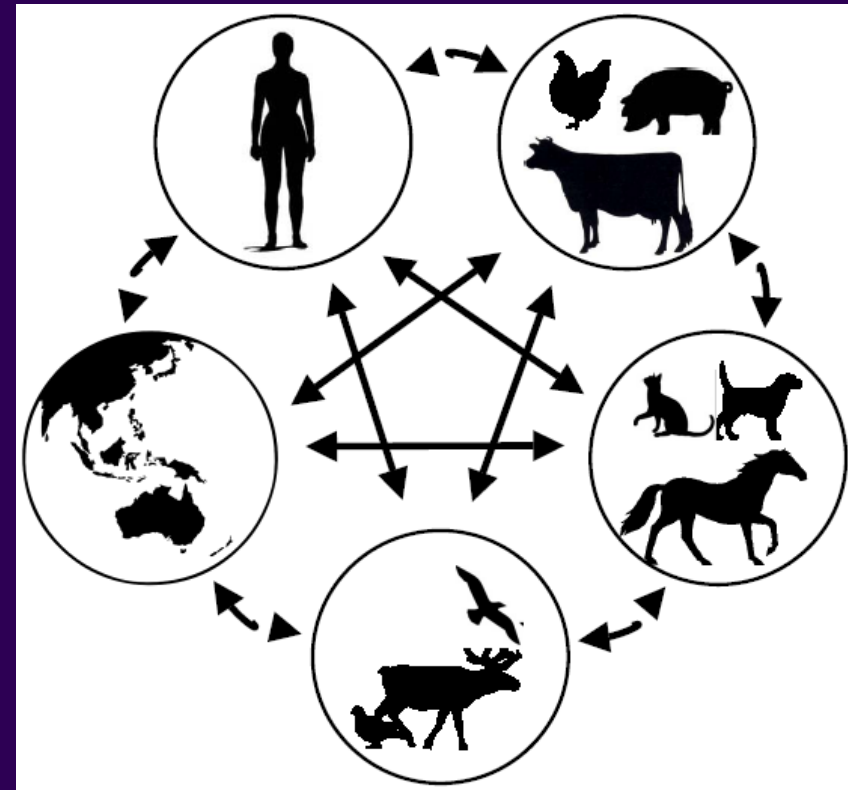


Success of the gene

- Good promoter
- Mobilizing agent (IS)
- Successful plasmid carrier

Success of the clone or strain

- Prevalence
- Virulence potential
- Locally, nationally or internationally
- Often poorly understood



Stokes & Gillings, FEMS Microbiol Rev, 2011

ESBLs in *E. coli* from humans and poultry in the UK



	Humans	Broilers	Turkeys
CTX-M types	15 >> 3, 14	1 >> 3, 15	14 >> 1, 15, 55
Plasmid <i>rep</i> types	FII, F1B, I1	I1-y (CTX-M-1) A/C & P (CTX-M-3)	I1-y (CTX-M-1) K (CTX-M 14) F (CTX-M1/14/15)
Major CTX-M-15 clone	ST131	ST156	ST156

- some genes / plasmid types are common to humans and poultry
- however, differences outweigh the similarities
- current risk to UK public health caused by poultry reservoir appears small; human reservoir has greater importance in the UK

...,but that could change; experience in The Netherlands is different

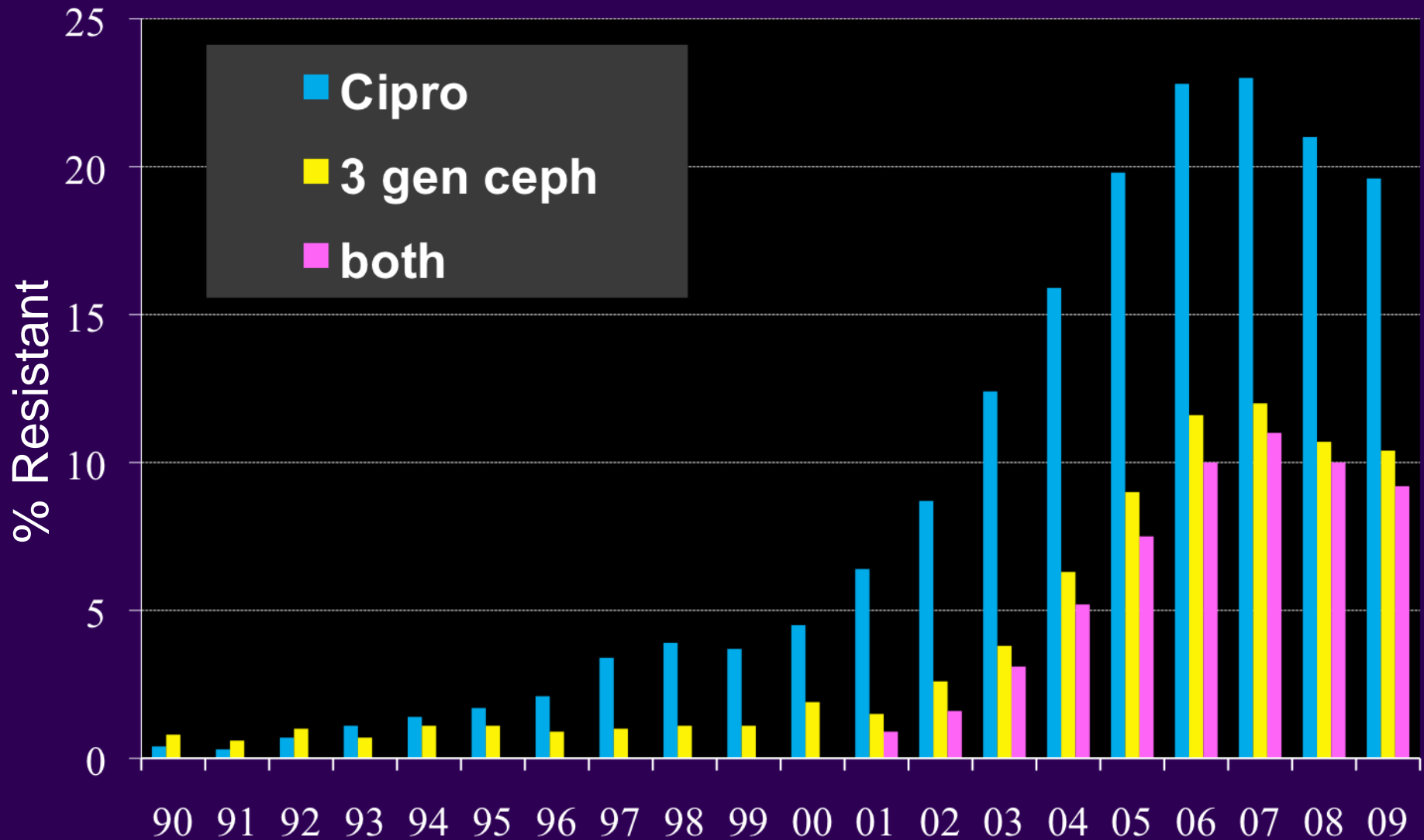


Level of genetic typing	% of human isolates with poultry associated genetic element ^a
ESBL genes (<i>bla</i> _{CTX-M-1} , <i>bla</i> _{TEM-52} , <i>bla</i> _{SHV-12} , <i>bla</i> _{SHV-2} and <i>bla</i> _{CTX-M-2})	35% (see Table 1)
<i>bla</i> _{CTX-M-1} and <i>bla</i> _{TEM-52} genes	30% (23.7% <i>bla</i> _{CTX-M-1} ; 6.2% <i>bla</i> _{TEM-52})
<i>bla</i> _{CTX-M-1} and <i>bla</i> _{TEM-52} genes on IncI plasmid	20% (14.2% <i>bla</i> _{CTX-M-1} ; 6.2% <i>bla</i> _{TEM-52})
<i>bla</i> _{CTX-M-1} and <i>bla</i> _{TEM-52} genes on IncI plasmid belonging to complex CC7 or CC3 and CC5 resp.	19% (12.6% <i>bla</i> _{CTX-M-1} ; 6.2% <i>bla</i> _{TEM-52})
<i>bla</i> _{CTX-M-1} and <i>bla</i> _{TEM-52} genes on IncI plasmid belonging to complex CC7 or CC3 and CC5 resp. in a poultry-associated MLST strain (ST10, ST58 or ST117)	11% (9.5% <i>bla</i> _{CTX-M-1} ; 2.0% <i>bla</i> _{TEM-52})

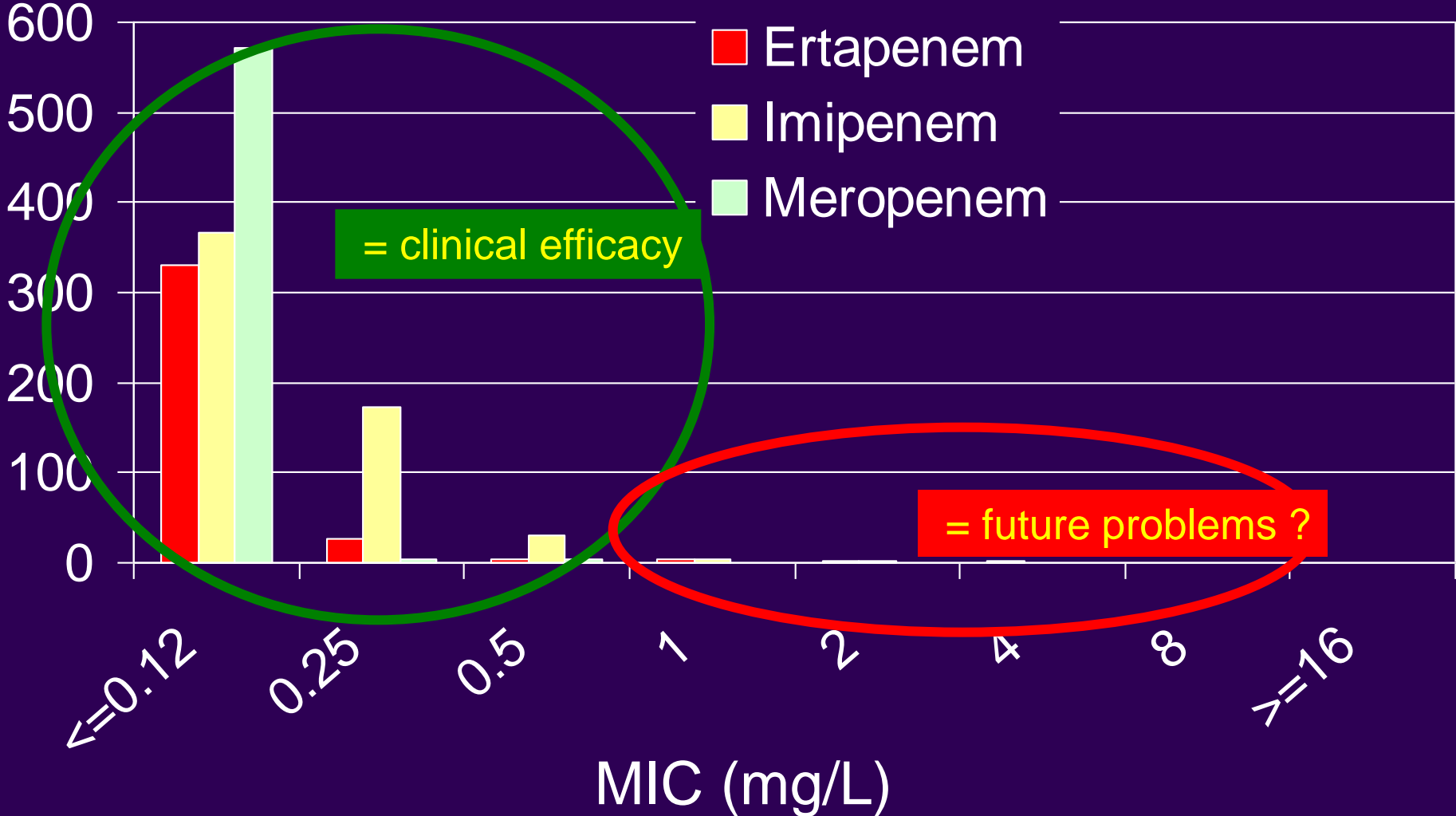
94% retail poultry samples were ESBL +ve

- c. 1 in 3 patients had a poultry-associated (PA) ESBL
- c. 1 in 5 patients had a PA ESBL encoded by a PA plasmid (pMLST)
- c. 1 in 10 patients had a PA ESBL encoded by a PA plasmid in a PA *E. coli* strain

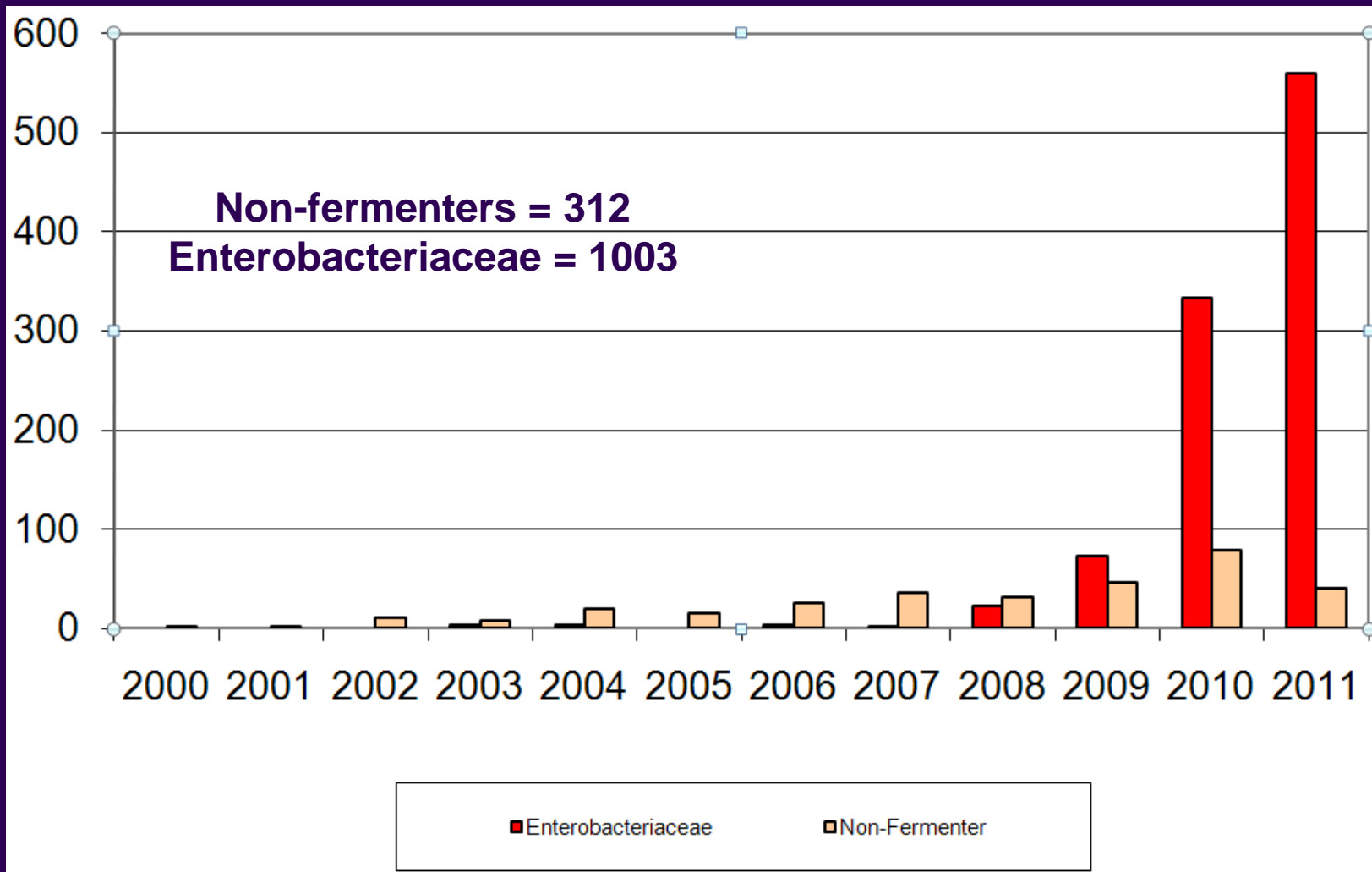
E. coli from blood & CSF in the UK - a recent fall in resistance



Carbapenems vs. ESBL +ve *E. coli*



Carbapenemase-mediated resistance in the UK (n = 1315)*



*excludes *A. baumannii* with OXA-types

ARMRL, Unpublished data

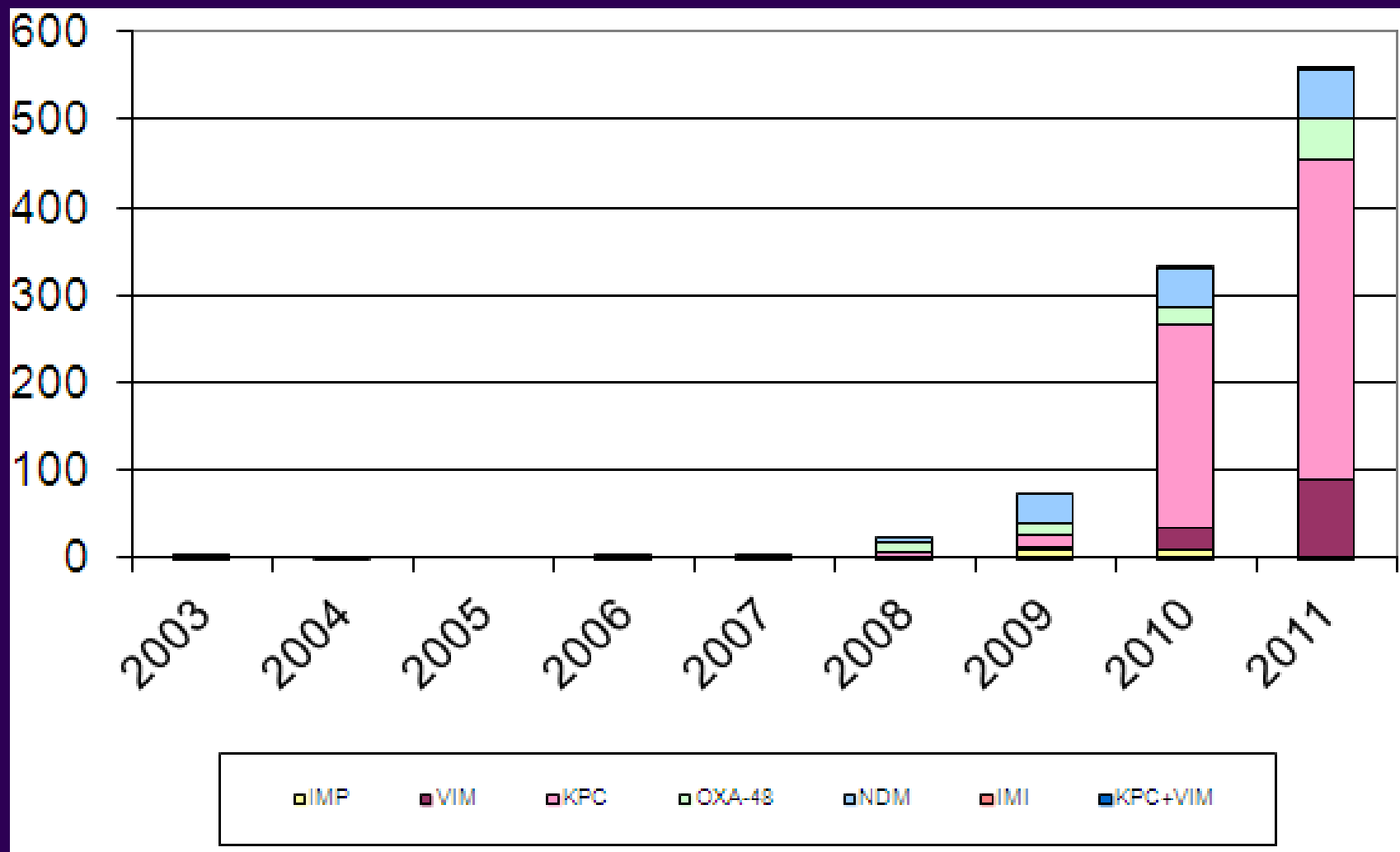
Acquired carbapenemases



Class	Carbapenemase	Enterobacteriaceae	Non-fermenters
A (non-metallo)	KPC	+++	+
	IMI, NMC, SME	+	-
B (metallo)	IMP*, VIM*	+++	+++
	NDM	+++	++
	AIM, DIM, SIM, SPM, TMB	-	++
D (non-metallo)	OXA-48-like	+++	-
	OXA-23, -40, -58, -143	+/-	+++

- IMP- & VIM- types are integron-associated
- IMP-types described first, but have been overtaken by other types

Carbapenemase-producing Enterobacteriaceae in the UK (n = 1003)

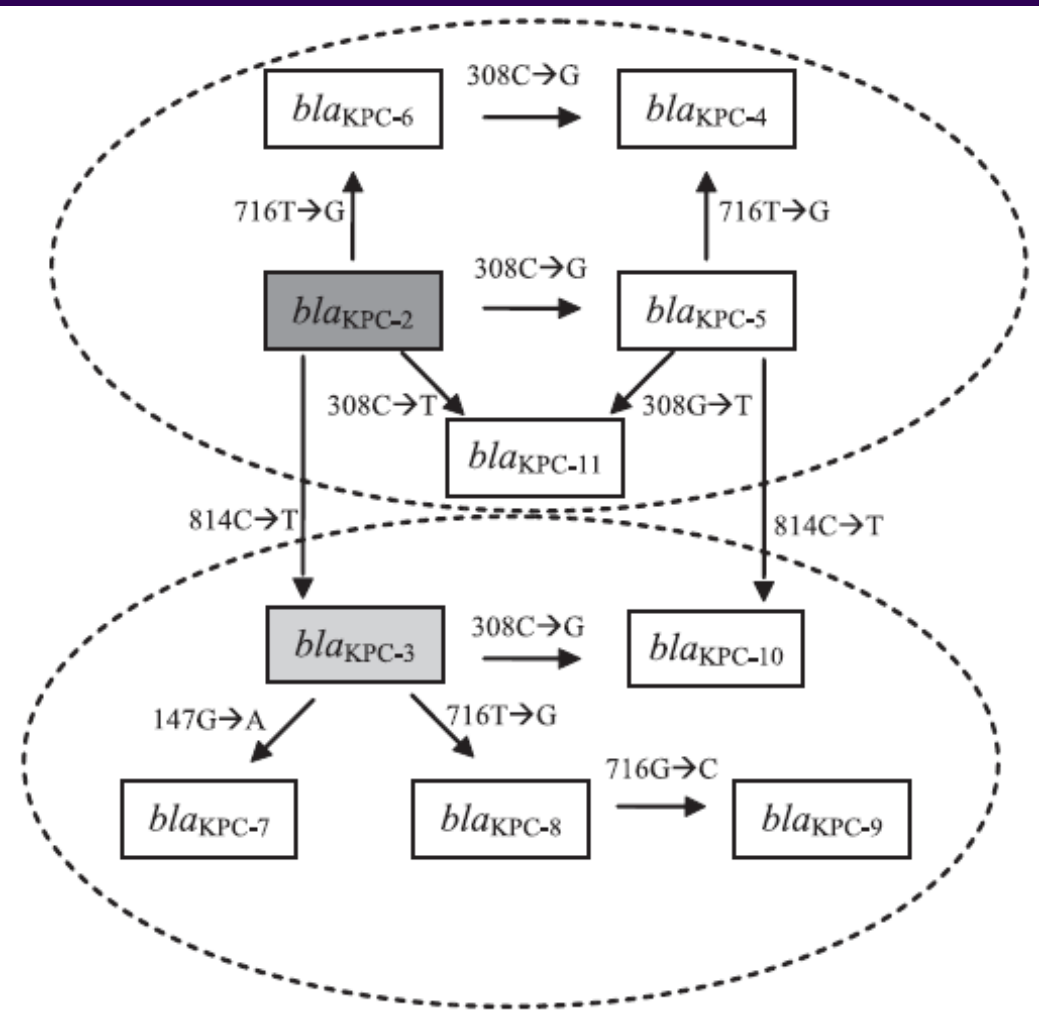


bla_{KPC} gene	KPC enzyme	Species	Yr isolated	Location	GenBank accession no.	Reference(s)
$bla_{\text{KPC-1}}$ ^b	KPC-1 ^b	<i>Klebsiella pneumoniae</i>	1996	North Carolina	AF297554	48
$bla_{\text{KPC-2}}$	KPC-2	<i>K. pneumoniae</i>	1998–1999	Maryland	AY034847	36
$bla_{\text{KPC-3}}$	KPC-3	<i>K. pneumoniae</i>	2000–2001	New York	AF395881	46
$bla_{\text{KPC-4}}$	KPC-4	<i>Enterobacter cancerogenus</i>	2003	Scotland	AY700571	27
$bla_{\text{KPC-5}}$	KPC-5	<i>Pseudomonas aeruginosa</i>	2006	Puerto Rico	EU400222	44 and 45
$bla_{\text{KPC-6}}$	KPC-6	<i>K. pneumoniae</i>	2003	Puerto Rico	EU555534	33
$bla_{\text{KPC-7}}$	KPC-7	<i>K. pneumoniae</i>	2007–2008	Ohio	EU729727	30
$bla_{\text{KPC-8}}$	KPC-8	<i>K. pneumoniae</i>	2008	Puerto Rico	FJ234412	15
$bla_{\text{KPC-9}}$	KPC-9	<i>Escherichia coli</i>	2009	Israel	FJ624872	Unpublished
$bla_{\text{KPC-10}}$	KPC-10	<i>Acinetobacter baumannii</i>	2009	Puerto Rico	GQ140348	31
$bla_{\text{KPC-11}}$	KPC-11	<i>K. pneumoniae</i>	2010		HM066995	Unpublished

^a Species, year, and location of initial report for each variant.

^b $bla_{\text{KPC-1}}$ and KPC-1 are no longer considered valid designations, as their sequences are identical to those of $bla_{\text{KPC-2}}$ and KPC-2, respectively (47).

Evolution of the KPC family



KPC-2 / -3 most prevalent types

Differ by His(272)-Tyr change

What selects this substitution ?

KPC +ve bacteria in the UK (Jan '12)



- 621 x Enterobacteriaceae

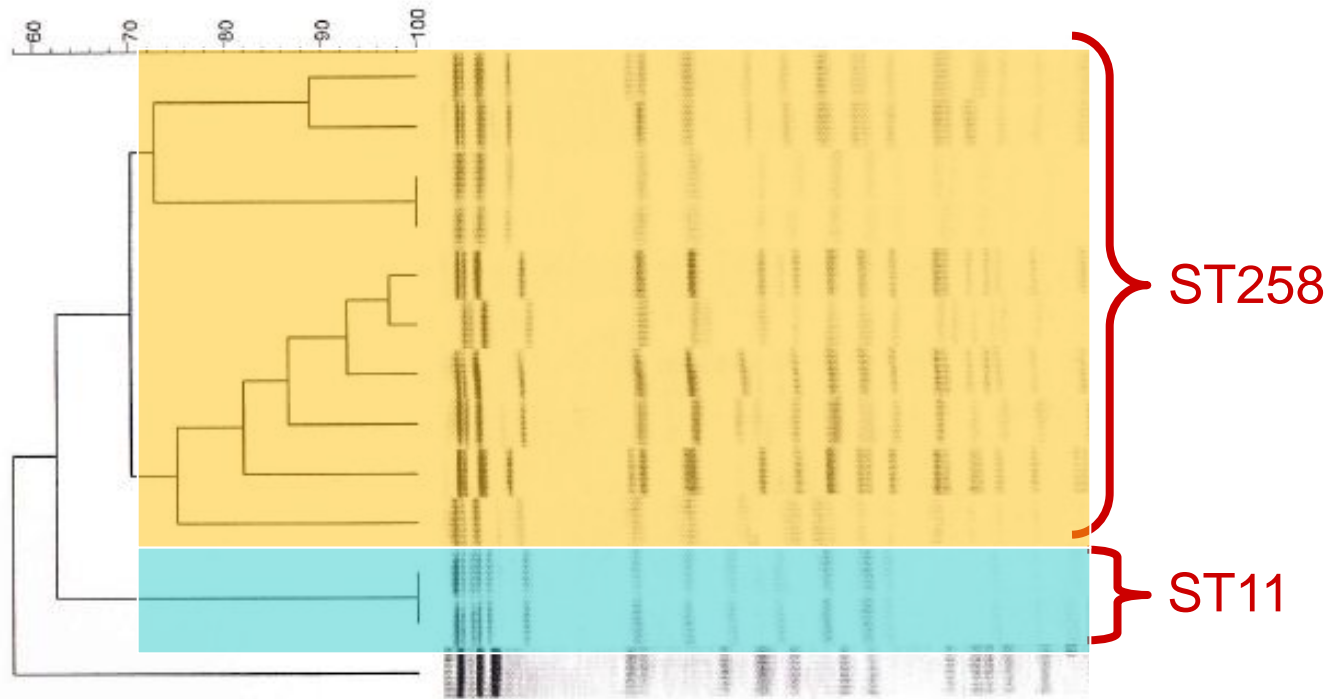
- 520 x *Klebsiella* spp.
- 50 x *E. coli*
- 40 x *Enterobacter* spp.
- 9 x *Raoutella* spp.
- 1 x *Citrobacter* spp.
- 1 x *Serratia* sp.

**Scattered, but dominant in
NW England**

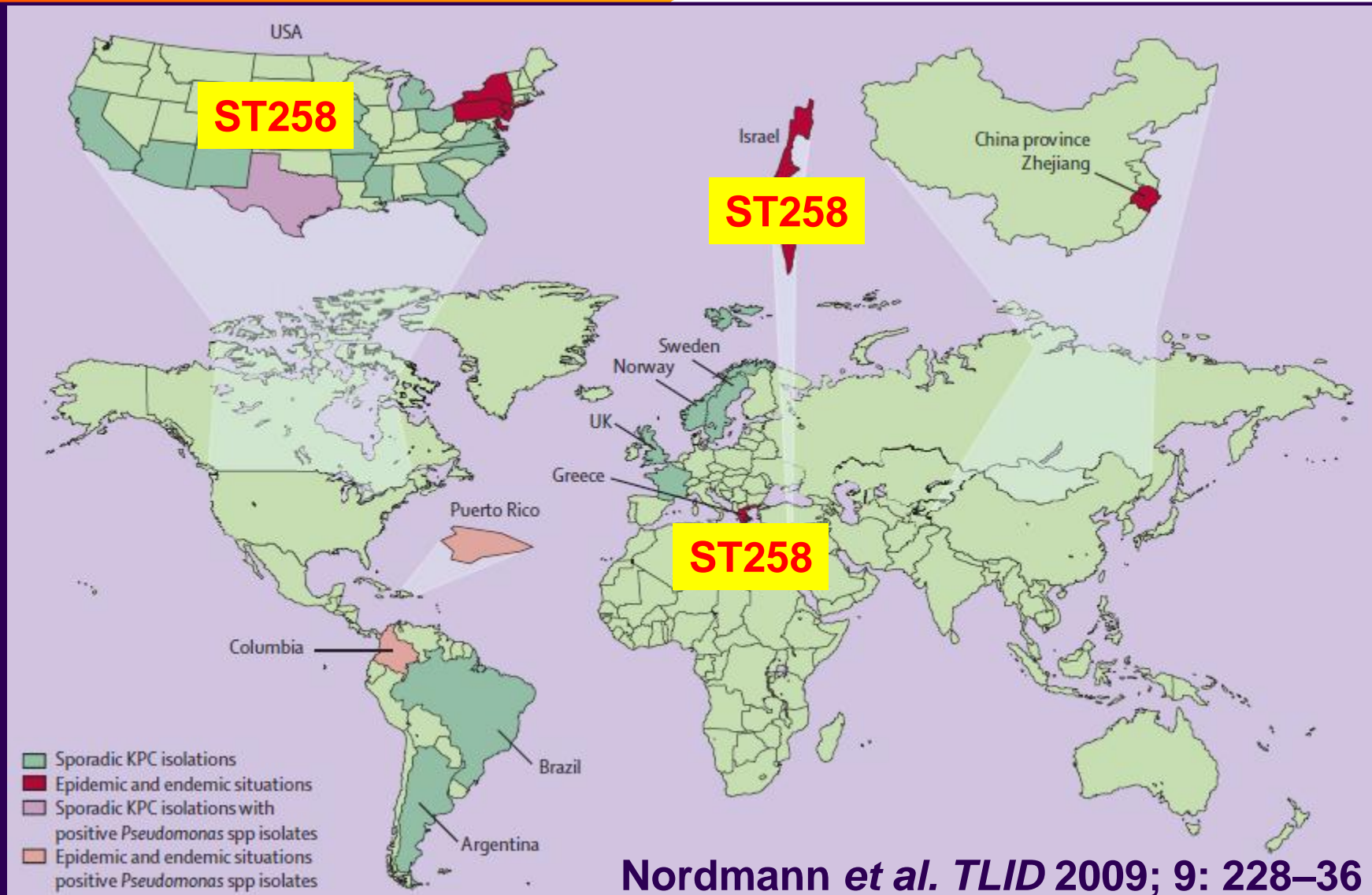
KPC-producing *K. pneumoniae* in the UK ...the first arrivals



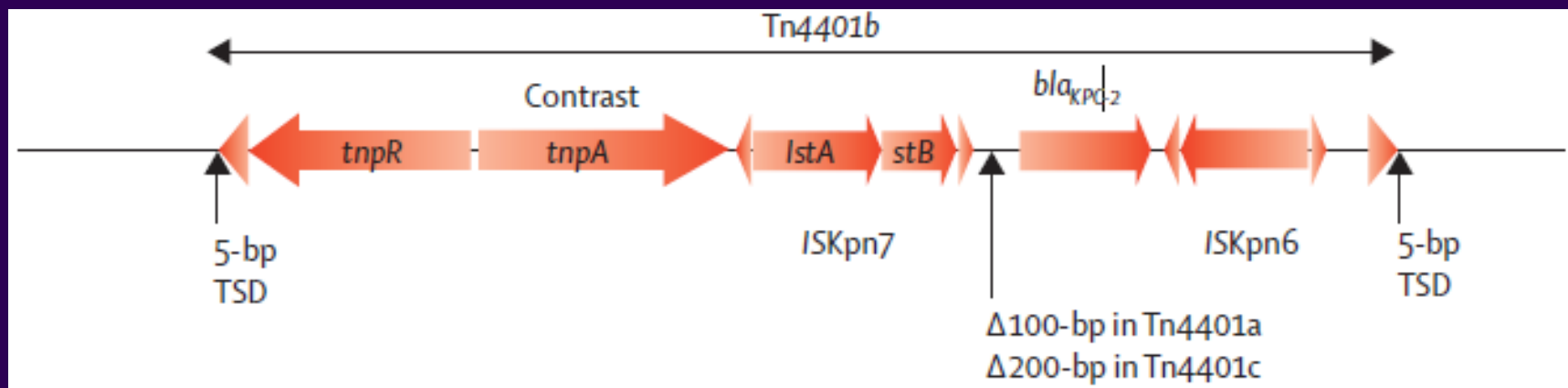
Dice (Opt: 1.00%) (Tol 1.0%-1.0%) (H>0.0% S>0.0%) [0.0%-100.0%]
PFGE PFGE



Strain dynamics: a dominant international KPC +ve *K. pneumoniae* clone



*bla*_{KPC} often on Tn4401 elements



- Various isoforms of this transposon
- May have *bla*_{KPC} -2 or -3

OXA-48-like carbapenemases: similar genes, but different plasmids



Endemic in Turkey.

Many European cases linked to North Africa, but few prevalence data for this region

One of current concerns for Libyan trauma victims

OXA-48-like enzymes in the UK (Jan '12)

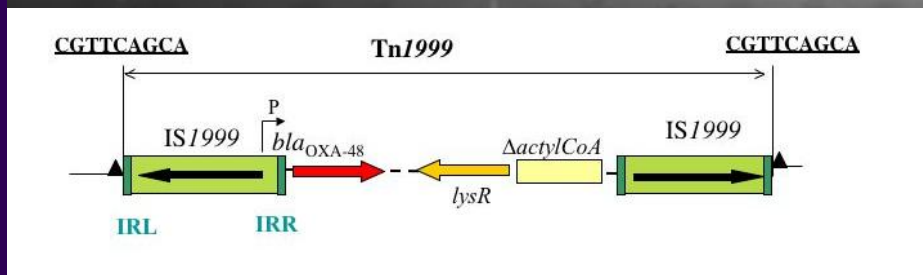
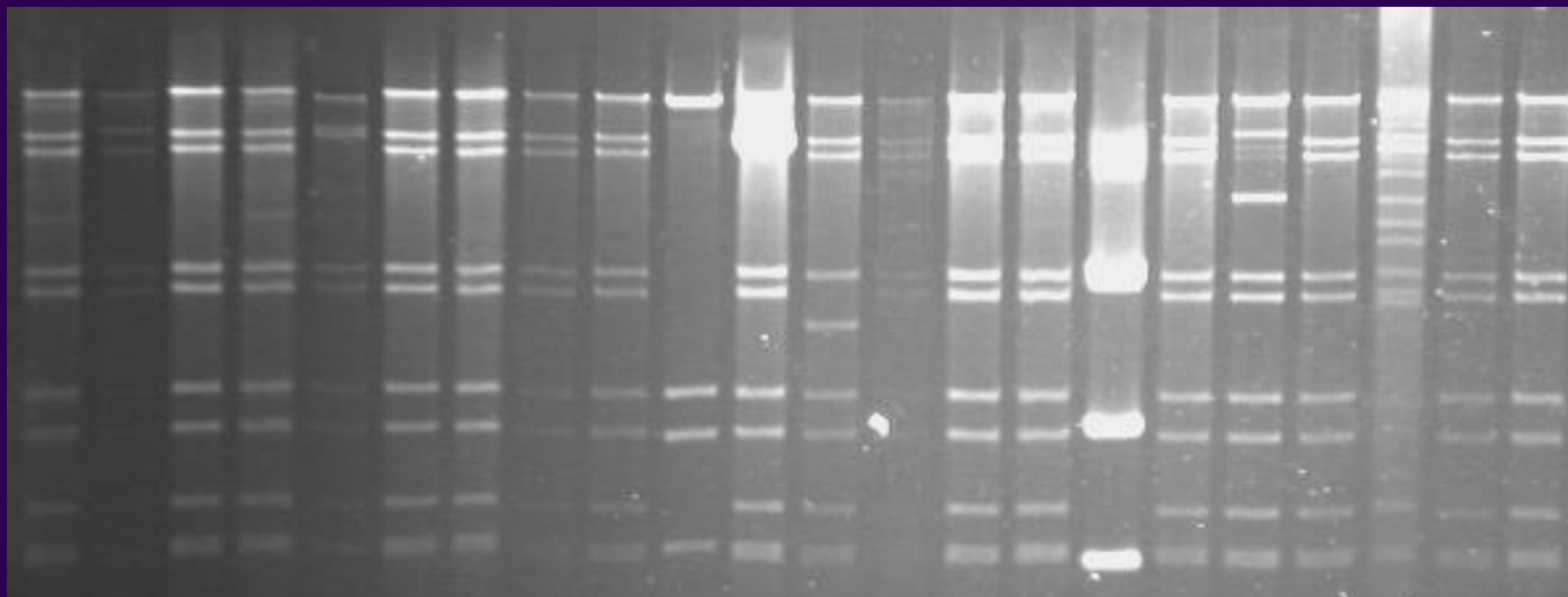
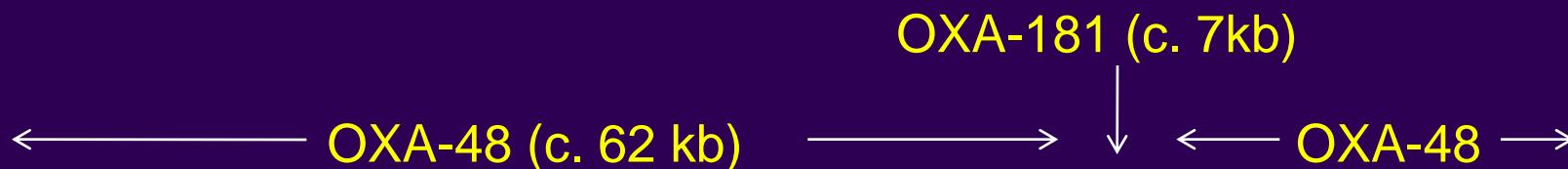


- 92 x Enterobacteriaceae

- 72 x *Klebsiella* spp.
- 17 x *E. coli*
- 2 x *Enterobacter* spp.
- 1 x *Citrobacter freundii*

Spread of highly related plasmids

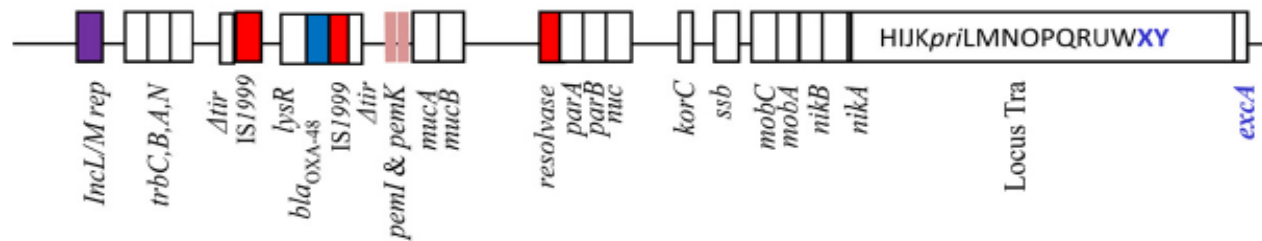
International plasmid 'epidemic': OXA-48 plasmids in *Klebsiella*, *Enterobacter* and *E. coli*



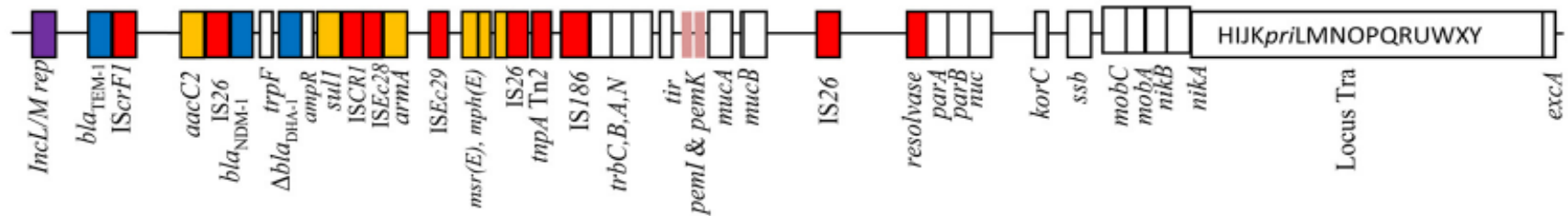
pOXA-48; ...new primers for PBRT



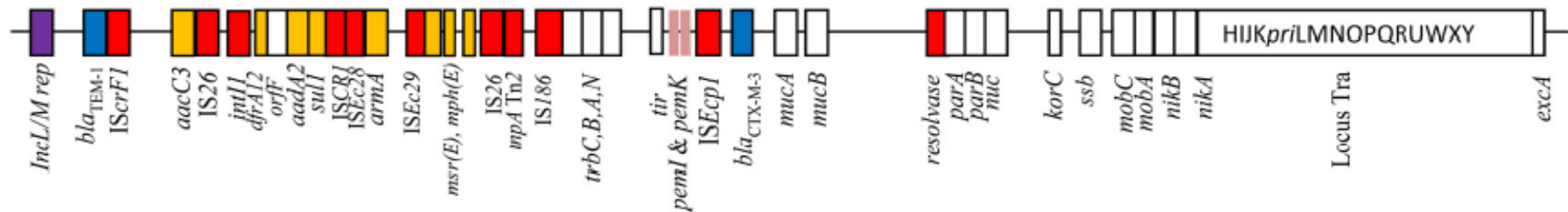
IncL/M pOXA-48, *Klebsiella pneumoniae*, 61,881 bp



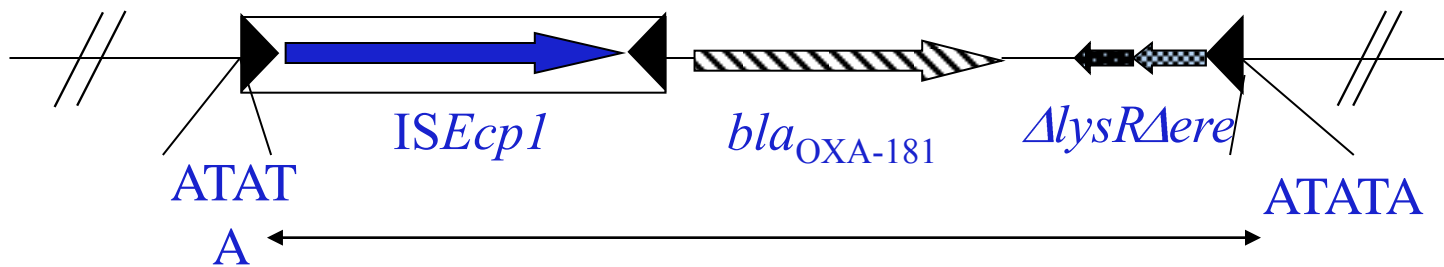
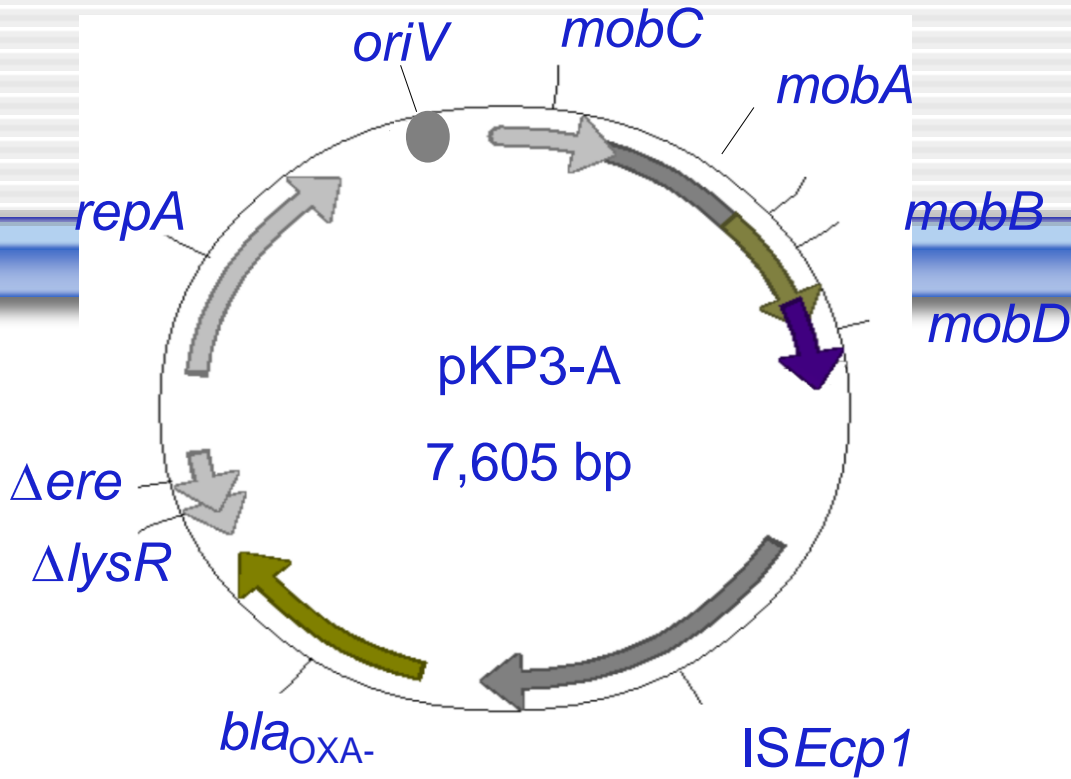
IncL/M pNDM-HK *Escherichia coli*, Honk-Kong, 88,803 bp, HQ451074



IncL/M pCTX-M-3, *Citrobacter freundii*, Poland, 89,468 bp, AF550415



OXA-181 carbapenemase

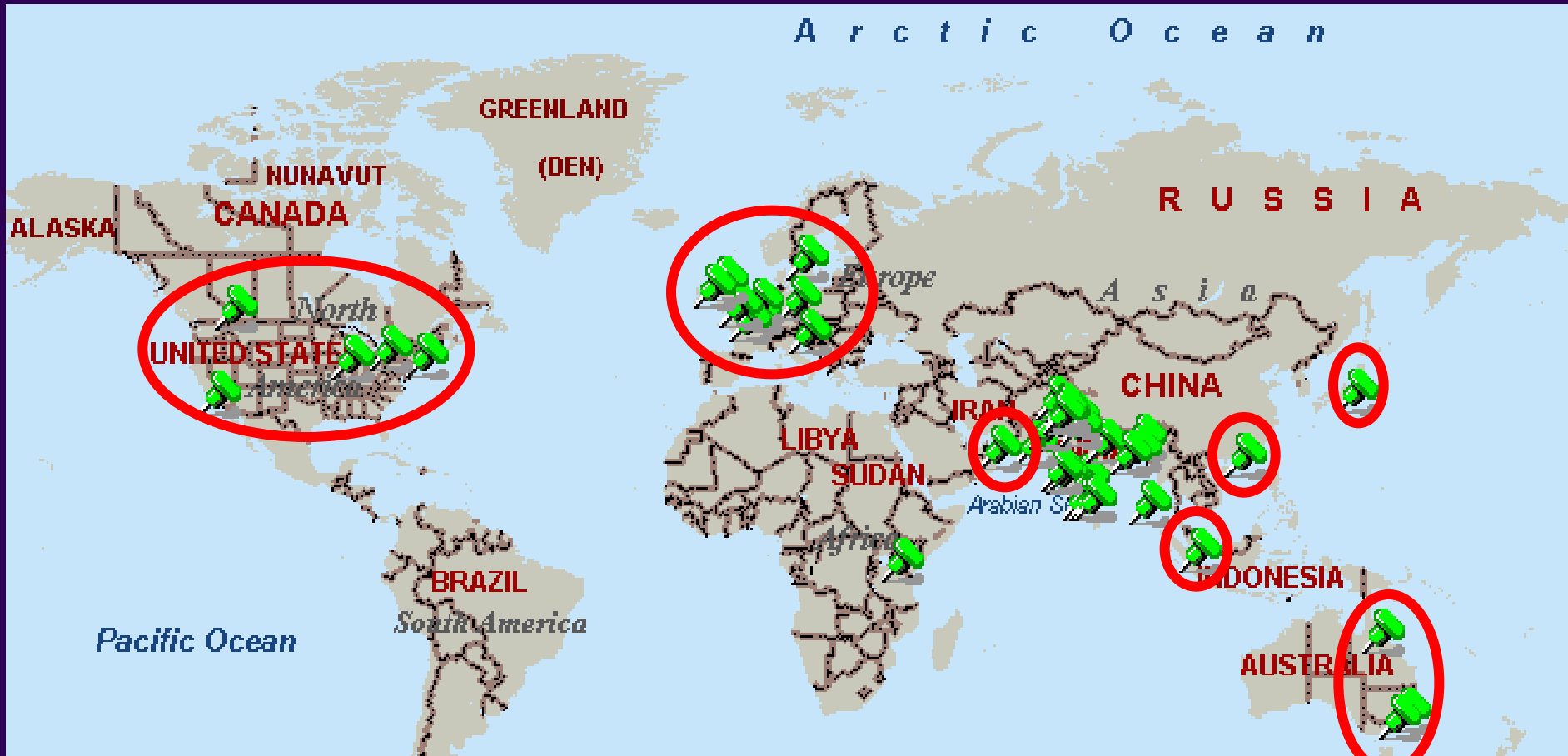


Diverse host strains: variable *bla* and resistance profiles



Isolate	Hospital + patient	Year	Isolation site	Travel History	ST	Carba-penemase	Other β -lactamase genes detected by PCR	MIC (mg/L) ^a				
								IPM	MEM	ETP	CTX	CAZ
<i>K. pneumoniae</i>												
KP01	A1	2007	Urine	Turkey	147	OXA-48	CTX-M group 1; OXA-1; TEM; SHV ^b	16	8	>16	>256	256
KP02 ^c	B2	2008	Wound	Unknown	353	OXA-48	TEM; SHV	4	2	>16	64	4
KP03	C3	2009	Urine	Unknown	432	OXA-48	OXA-1; SHV	4	2	8	1	0.25
KP04	D4	2009	Urine	Unknown	101	OXA-48	CTX-M group 1; OXA-1; TEM; SHV	2	0.5	4	256	256
KP05	E5	2009	Fluid	Unknown	383	OXA-48	CTX-M group 1; OXA-1; SHV	2	1	16	>256	256
KP07	F6	2010	Urine	Unknown	14	OXA-48	CTX-M group 1; SHV	4	1	8	>256	>64
KP08	G7	2010	Blood	Unknown	858	OXA-48	CTX-M group 1; OXA-1; TEM; SHV	2	0.5	4	256	64
KP09	H8	2010	Urine	None	17	OXA-48	OXA-1; TEM; SHV	2	2	8	1	0.5
KP16	I9 ^d	2010	Urine	Turkey	152	OXA-48	SHV	2	2	16	8	1
KP17	J10	2011	Fluid	Unknown	101	OXA-48	CTX-M group 1; OXA-1; TEM; SHV	2	2	8	>256	256
KP20	K11	2011	Blood	Unknown	101	OXA-48	CTX-M group 1; OXA-1; OXA-9; TEM; SHV	8	16	>16	>256	128
KP22	E12	2011	Wound	Pakistan	37	OXA-48	CTX-M group 1; OXA-1; SHV	2	8	8	256	>256
KP24	L13	2011	Urine	Unknown	376	OXA-48	CTX-M group 9; OXA-9; TEM; SHV	8	8	>16	>256	64
KP19	B14	2011	Blood	India	11	OXA-181	CTX-M group 1; OXA-1; SHV	128	32	>16	>256	64
<i>E. coli</i>												
EC06	H15	2010	Wound	Unknown	648	OXA-48	CTX-M group 1; OXA-1	1	0.5	4	>256	32
EC10	M16	2010	Blood	None	131	OXA-48	None	2	1	8	4	0.25
EC11	B17	2010	Wound	Unknown	38	OXA-48	CTX-M group 9; TEM	4	8	>16	256	8
EC12	N18	2010	Urine	Unknown	10	OXA-48	TEM	4	0.5	2	2	0.25
EC13	J19	2010	Blood	Unknown	38	OXA-48	CTX-M group 9; TEM	64	>32	>16	256	4
EC15	O20	2010	Urine	Unknown	155	OXA-48	TEM	4	0.5	4	0.5	0.25
EC18	P21	2011	Urine	Unknown	38	OXA-48	CTX-M group 9; TEM	2	0.5	4	256	1
EC21	Q22	2011	Urine	Unknown	88	OXA-48	TEM	8	4	16	8	0.5

NDM carbapenemases: global reports, but a clear epicentre



Many cases with travel links / hospitalization in sub-continent
Some link to the Balkans ...2nd epicentre ?

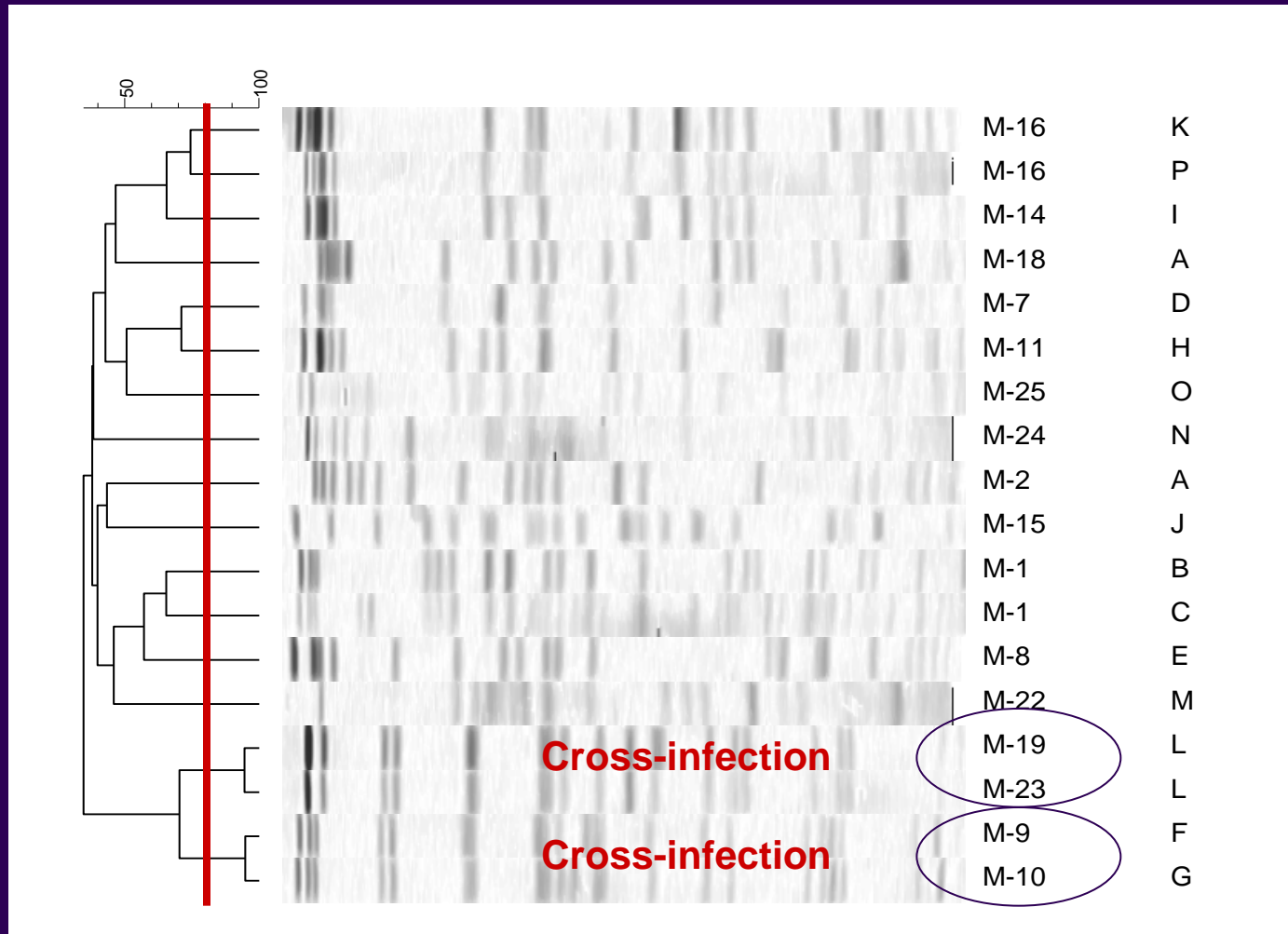
NDM +ve bacteria in the UK (115 patients, Jan '12)



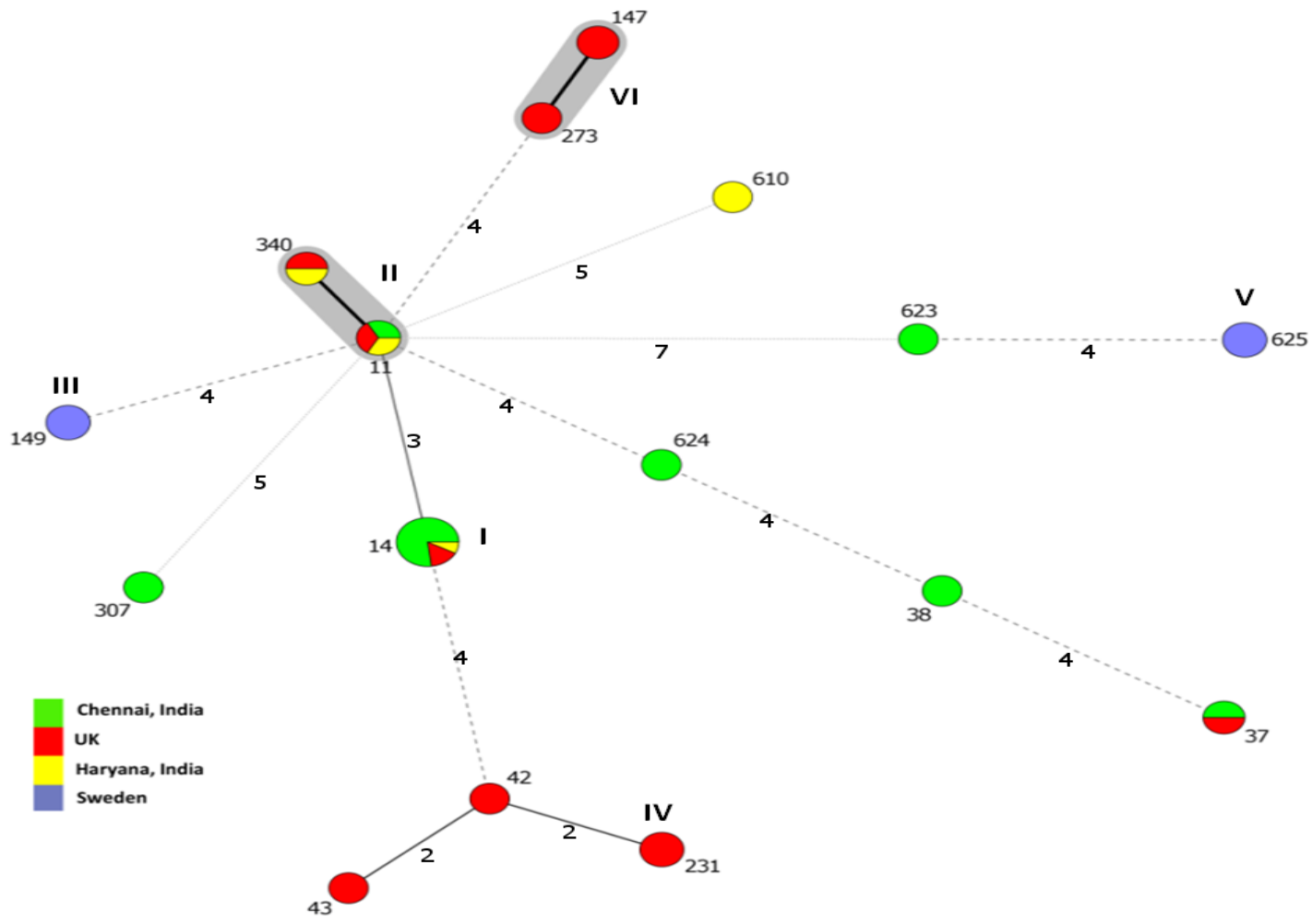
- 138 x Enterobacteriaceae
 - 87 x *Klebsiella* spp.
 - 33 x *E. coli*
 - 12 x *Enterobacter* spp.
 - 3 x *Citrobacter* spp.
 - 1 x *Morganella morgannii*
 - 1 x *Providencia* sp.
 - 1 x *Serratia* sp.
- 11 x *A. baumannii*

NDM-1 is coded by a mobile gene, on mobile plasmids, and will spread further

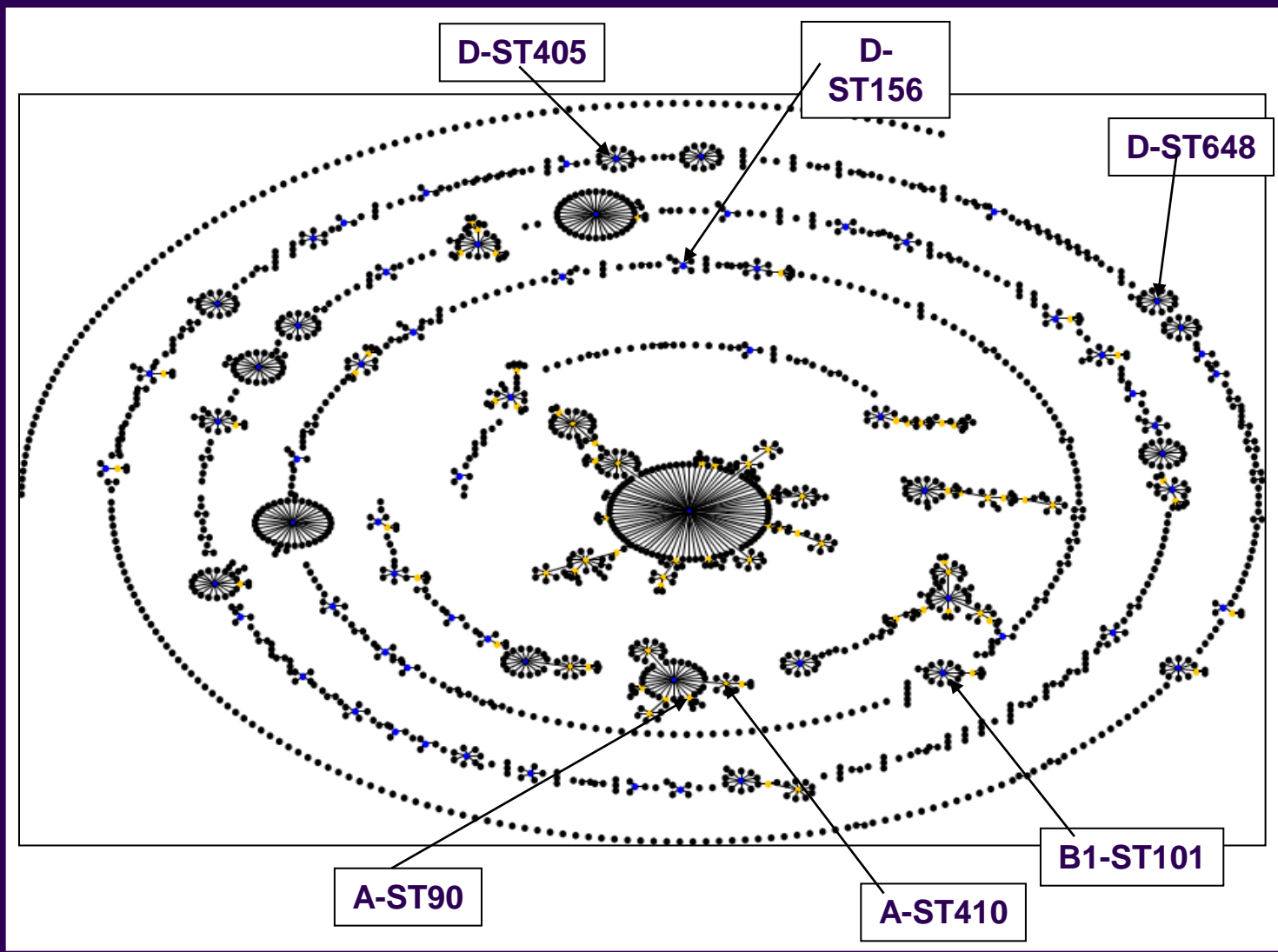
NDM +ve *K. pneumoniae* in the UK are diverse



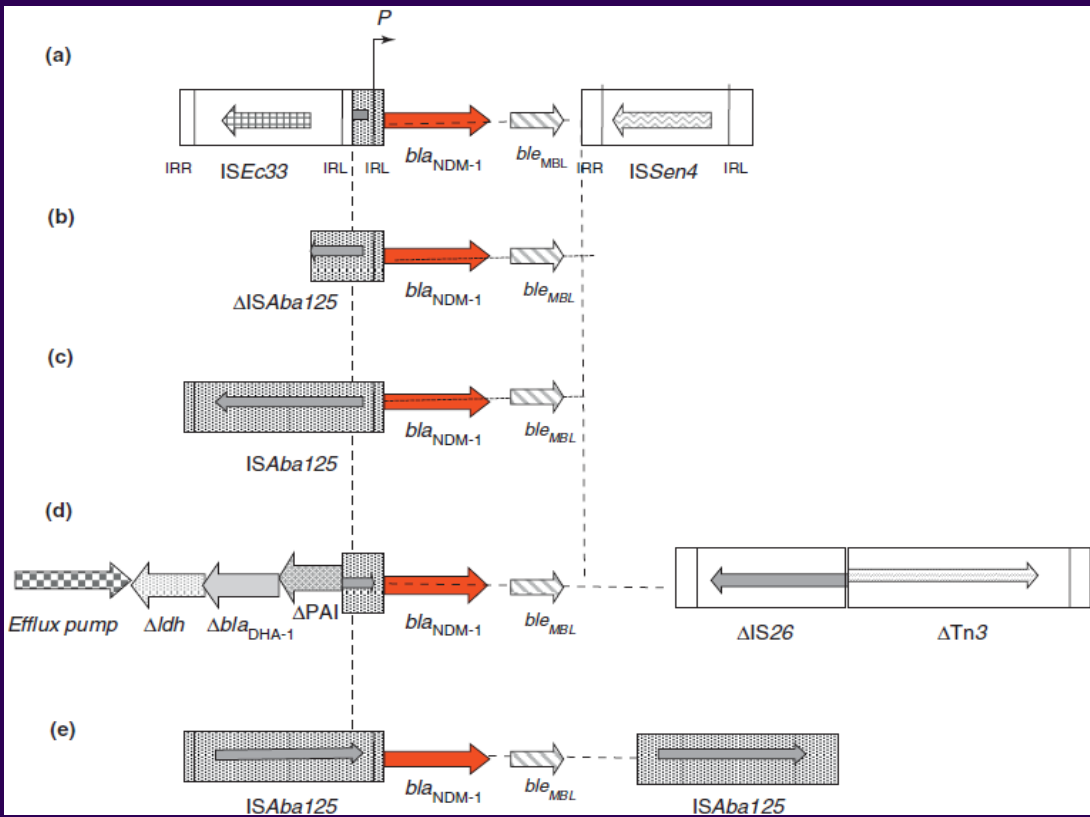
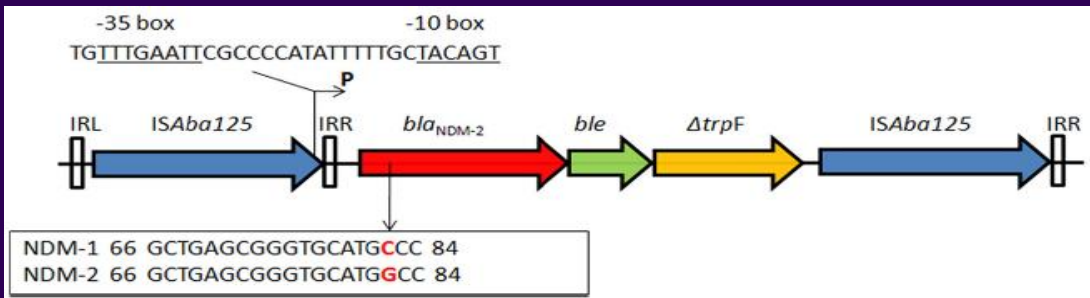
...as they are internationally



...and so are the *E. coli*



...and in diverse (but often related) genetic contexts



- intact or partial ISAbA125
 - provides promoter
- bleomycin resistance gene
- limited sources for the original escape event
- escape from 'species X' to *Acinetobacter* and then to Enterobacteriaceae

Antibiotic	Metallo-enzyme Producers (IMP, NDM or VIM)			Non-metallo-enzyme Producers (KPC or OXA-48-like)		
	<i>E. coli</i>	<i>Klebsiella</i>	<i>Enterobacter / Citrobacter</i>	<i>E. coli</i>	<i>Klebsiella</i>	<i>Enterobacter / Citrobacter</i>
Imipenem	9%	1%	3%	10%	5%	18%
IPM+EDTA [b]	100%	99%	100%	27%	8%	27%
Meropenem	9%	5%	3%	47%	8%	27%
Ertapenem	0%	0%	0%	0%	0%	0%
Ampicillin	0%	0%	0%	0%	0%	0%
Co-amoxiclav	0%	0%	0%	0%	0%	0%
Piperacillin	0%	0%	3%	0%	0%	0%
PIP + tazobactam	4%	0%	7%	0%	0%	0%
Cefotaxime	0%	0%	0%	3%	2%	0%
Ceftazidime	0%	0%	0%	17%	6%	0%
Aztreonam	4%	18%	13%	13%	6%	0%
Ciprofloxacin	9%	10%	17%	53%	49%	50%
Gentamicin	0%	12%	27%	70%	65%	41%
Tobramycin	0%	1%	0%	50%	58%	50%
Amikacin	17%	32%	50%	90%	85%	91%
Colistin	100%	97%	93%	100%	92%	100%
Tigecycline	100%	47%	47%	100%	74%	68%

a. Susceptibility defined using BSAC v. 10.1 breakpoints [7].

b. Diagnostic test to distinguish metallo- from non-metallo- enzymes; not for therapeutic use.

Active vs. ≥90% producers	Active vs. >75-89% producers
Active vs. 50-74% producers	Active vs. <50% producers

..., and resistance may emerge to the few active agents



Table 1. MICs in mg/L for NDM-1-producing *E. coli* isolated from the patient; the second was isolated from blood 4 months after the original was isolated from a calciphylactic skin lesion

Antimicrobial agent	Isolate 1 (calciphylactic lesion)	Isolate 2 (blood culture)
Amikacin	>64	>64
Gentamicin	>32	>32
Amoxicillin/clavulanate	64	64
Ampicillin	>64	>64
Aztreonam	>64	>64
Cefotaxime	>256	>256
Cefpirome	>64	>64
Ceftazidime	>256	>256
Ertapenem	>16	>16
Imipenem	64	64
Meropenem	>32	>32
Piperacillin/tazobactam	>64	>64
Colistin	<0.5	<0.5
Ciprofloxacin	>8	>8
Tigecycline	<0.25	8

Limiting the impact of carbapenemases



- **Detect resistance rapidly in the clinical laboratory**
 - Hodge tests / synergy tests / automated systems / agreed algorithms
 - Reference laboratory support
 - Molecular tests
- **Identify infected / colonized patients. Essential for :**
 - appropriate patient management
 - rapid implementation of infection control procedures
- **Prevent onwards transmission**

Advice on Carbapenemase Producers: Recognition, infection control and treatment

ARHAI

Department of Health
Advisory Committee on Antimicrobial Resistance
and Healthcare Associated Infection



Advice on Carbapenemase Producers:
Recognition, infection control and treatment

Reasons for the success of ESBLs and carbapenemases



- Multifactorial; highly complex; diverse
- Generalizations are overly simplistic
- Country-to-country variation in relative importance

	ESBLs	Carbapenemases
Successful host strains / clones	+++	+++
Successful plasmids	+++	+++
Community reservoirs (human)	+++	+
International human travel	+++	+++
Animal reservoirs	+++	+/- (?)
Food chain	+	- (?)

- What are the population structures of host species
 - Which STs dominate ? Need for structured surveillance
- Do distributions of carbapenemases reflect this?
 - getting into successful clones ?
 - or making 'lesser' clones successful ?
- Why is ST258 strongly associated only with KPC ?
- Relevance of His(272) → Tyr change in KPC-2 / -3 ?
- Reasons for stability of pOXA-48a plasmids ?

- **Better understanding of resistant bacterial clones**
 - distribution (global, national , regional)
 - contribution resistance plays to success
- **Better and faster diagnostics**
 - early, effective interventions
- **New treatment options**
 - our 'last resorts' are severely threatened