

# Activities of the National Reference Laboratory in UK



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**HPA - ARMRL - Colindale**

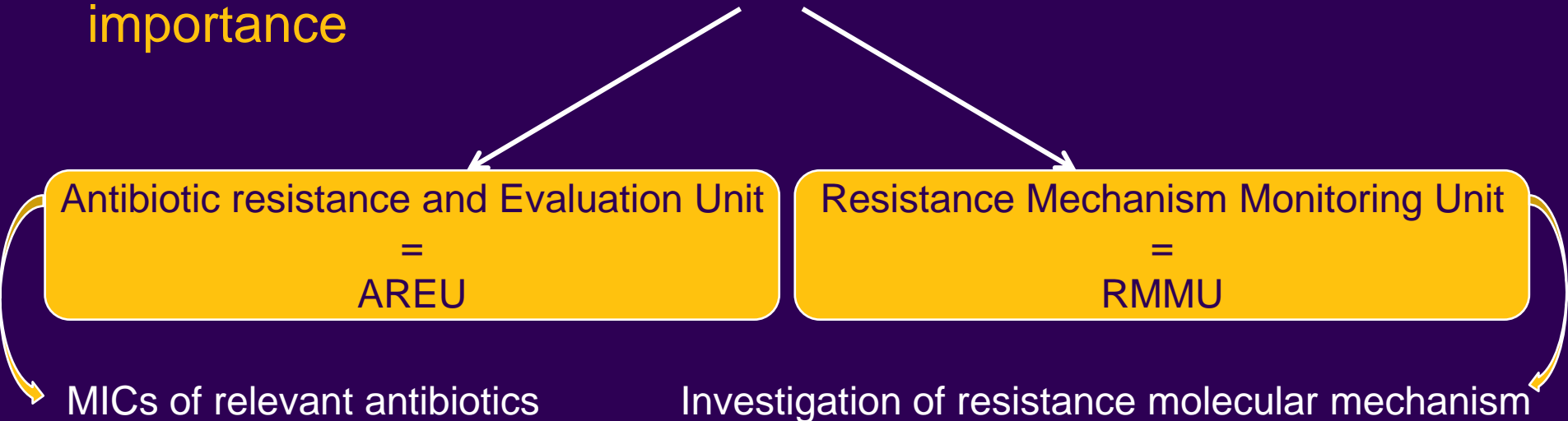


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# Antibiotic Resistance Monitoring and Reference Laboratory - ARMRL



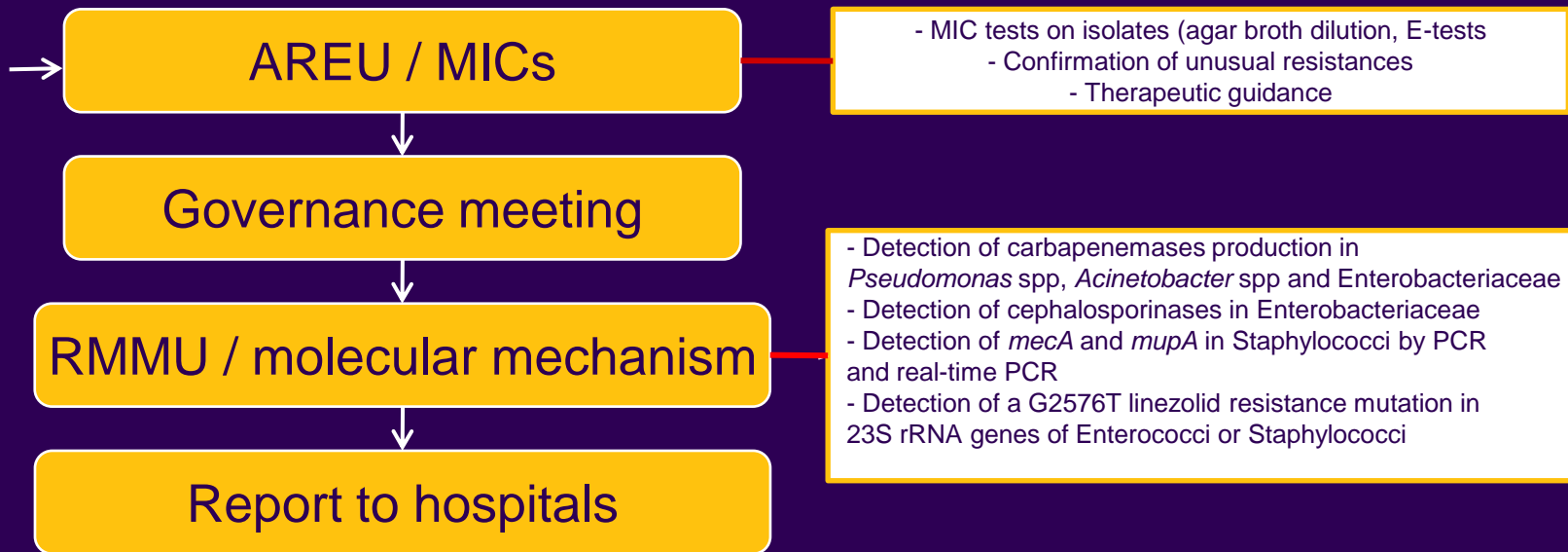
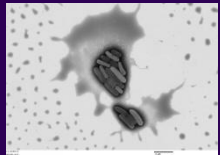
- National Reference Laboratory for the detection and investigation of antibiotic resistance of public health importance



# Workflow



~ 100 isolates/week



- MICs by agar dilution
- Interpretation against BSAC and EUCAST guidelines, inferring resistance mechanisms by interpretative reading
- MICs of a wide selection of relevant antibiotics for resistant isolates of any species within our remit
- MICs on antibiotics more suitable to detect resistance mechanisms (carbenicillin in *Pseudomonas*...)

# Current methods for the detection of antibiotic resistance genes



- Simplex PCR for Ambler class A (KPC, SME, IMI, AIM, KHM, GES) and class D (OXA-48) carbapenemases, class B metallo- $\beta$ -lactamase (NDM)
- Multiplex PCR for class B metallo- $\beta$ -lactamase (GIM, IMP, VIM, SIM, SPM)
- Multiplex PCR for plasmidic AmpCs (ACC, CIT, DHA, EBC, FOX, MOX)
- Real-time PCR for detection of MecA/MupA
- PCR-RFLP for linezolid

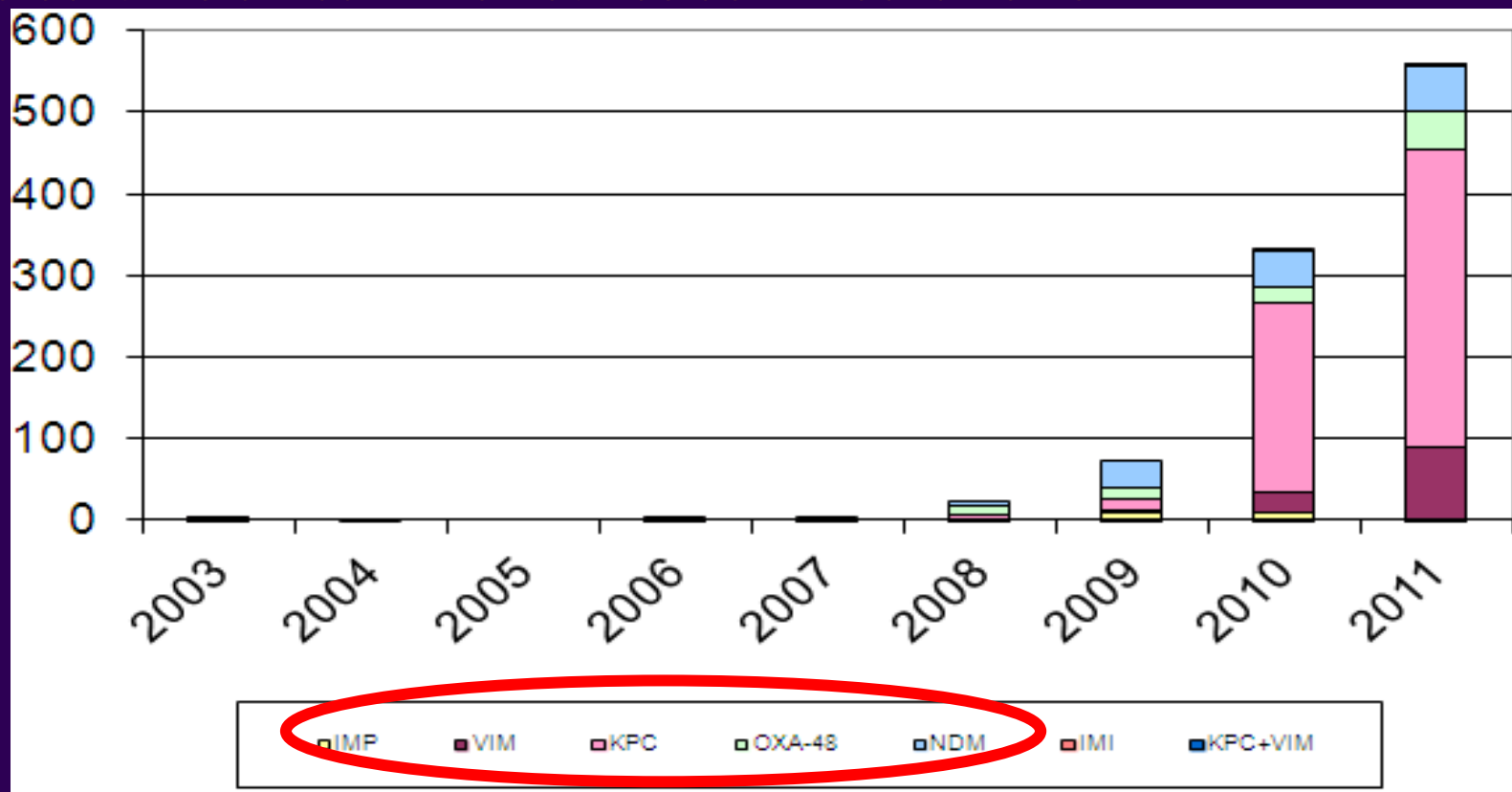


- Time consuming, turnaround times to be reduced
- Molecular tests done on ~ 30 isolates/wk, several tests (PCR) per isolate to confirm or refute presence of carbapenemases

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



- KPC, OXA-48, NDM, VIM and IMP : top 5 carbapenemases in UK
- Detection is difficult when co-resident mechanisms



# Need to improve our phenotypic detection methods



## Temocillin vs OXA-48

	MIC (mg/L)		
All species	32	64	128
OXA-48 +ve	4		23
OXA-48 -ve	24	13	23

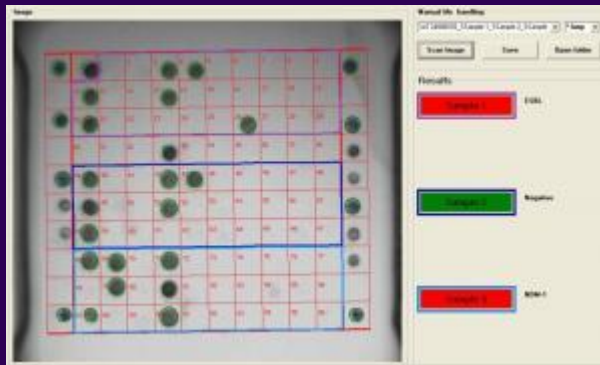
## Temocillin vs KPC

	MIC (mg/L)				
Species	8	16	32	64	128
<i>Klebsiella</i> spp	-	31	27	15	2
<i>E. coli</i>	1	8	6	-	-
<i>Enterobacter</i> spp.	1	-	2	1	-

# Need to improve our molecular detection methods



- Evaluation of DNA microarray Check-points MDR CT102 : detection of KPC, OXA-48, NDM-1, GIM, IMP and ESBLs (CTX-M group, SHV, TEM)



← One array per 3 samples

← In validation process

- Maldi-TOF : carbapenemase detection
- Luminex assay for detection of carbapenemases :  
multiplex PCR of the regions of interest + primer extension step specific of the allele





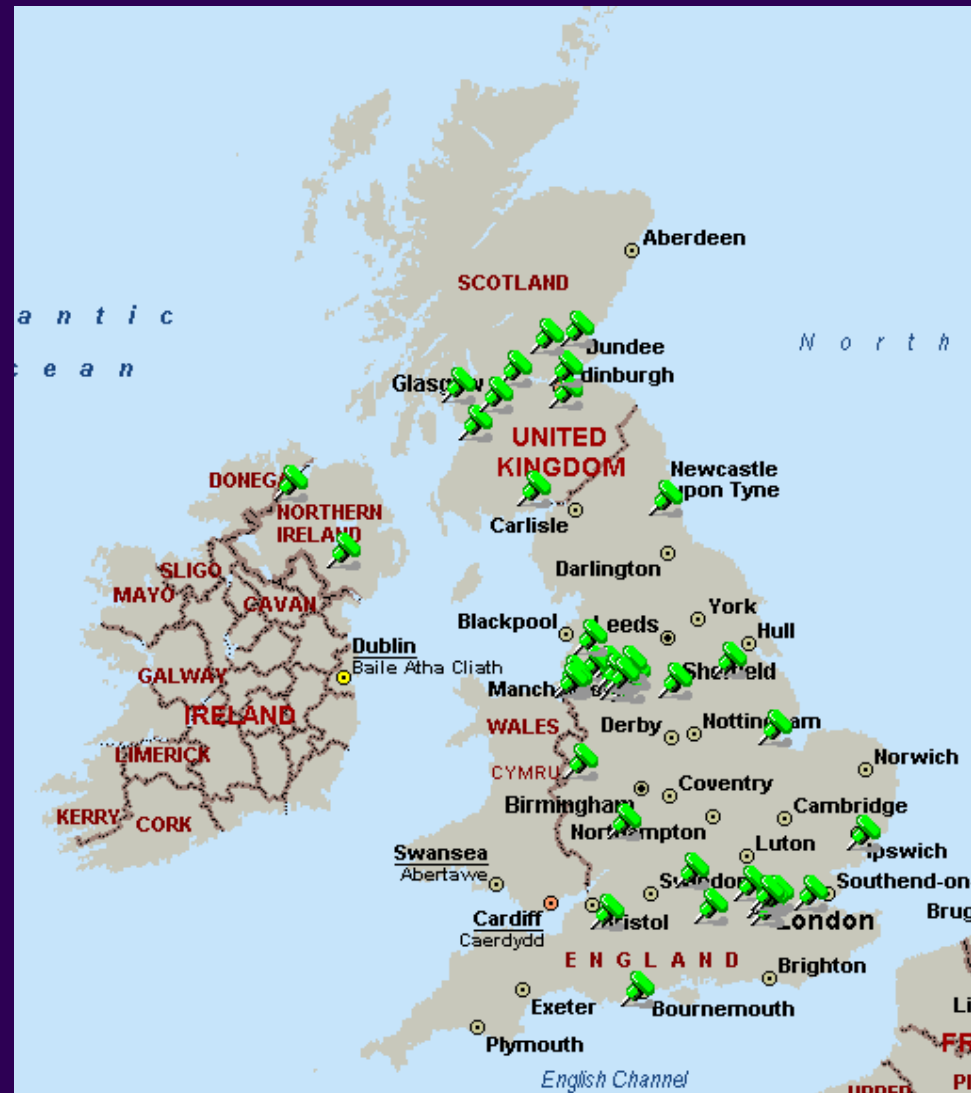
# KPC +ve bacteria in the UK (Jan '12)



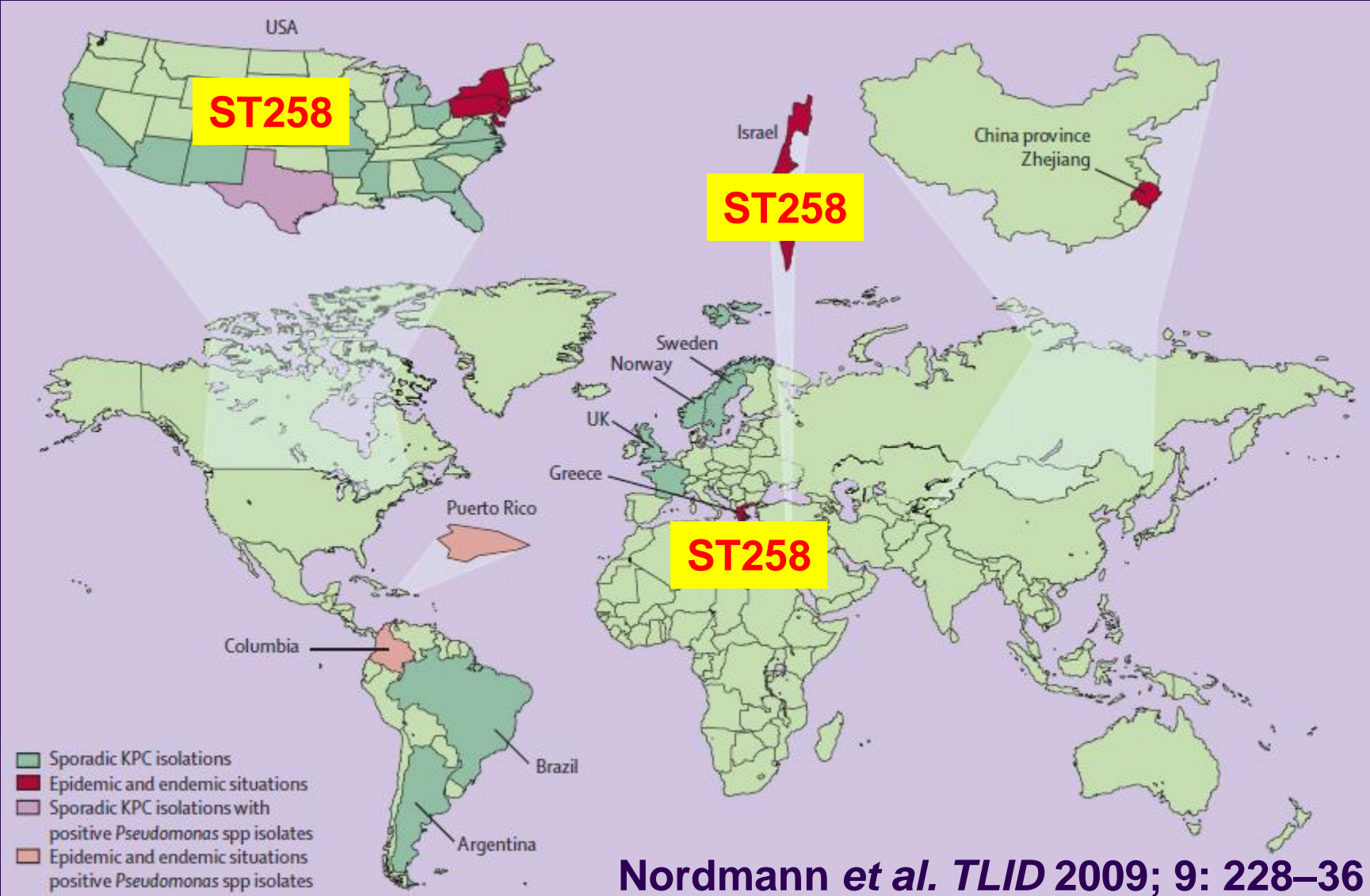
- 621 x Enterobacteriaceae

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

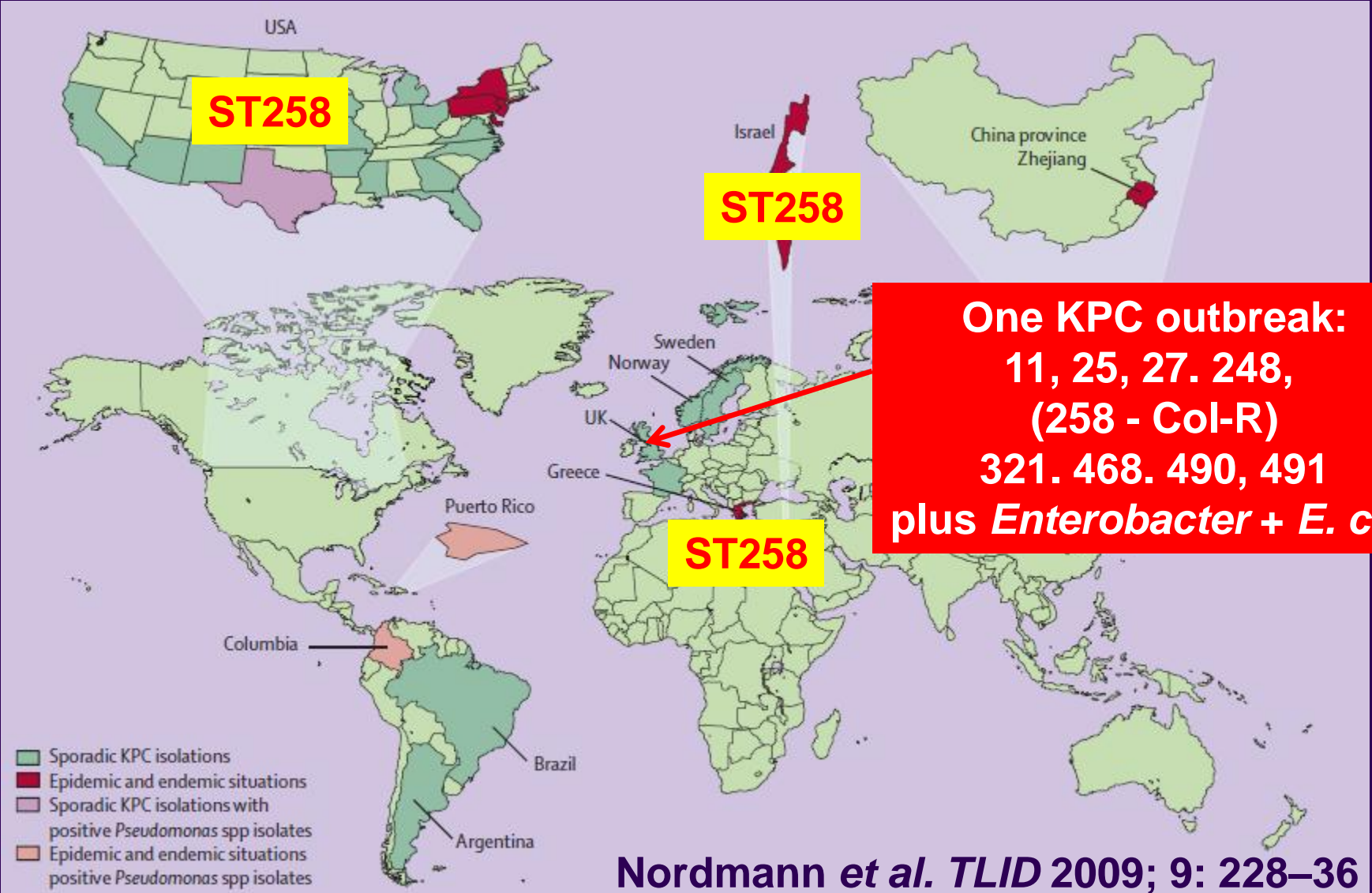
**Scattered, but dominant in  
NW England ...c. 75% of all  
isolates**



# International strain epidemic: KPC +ve *K. pneumoniae*



# ..., but in NW England it's different



**One KPC outbreak:  
11, 25, 27, 248,  
(258 - Col-R)  
321, 468, 490, 491  
plus *Enterobacter* + *E. coli***



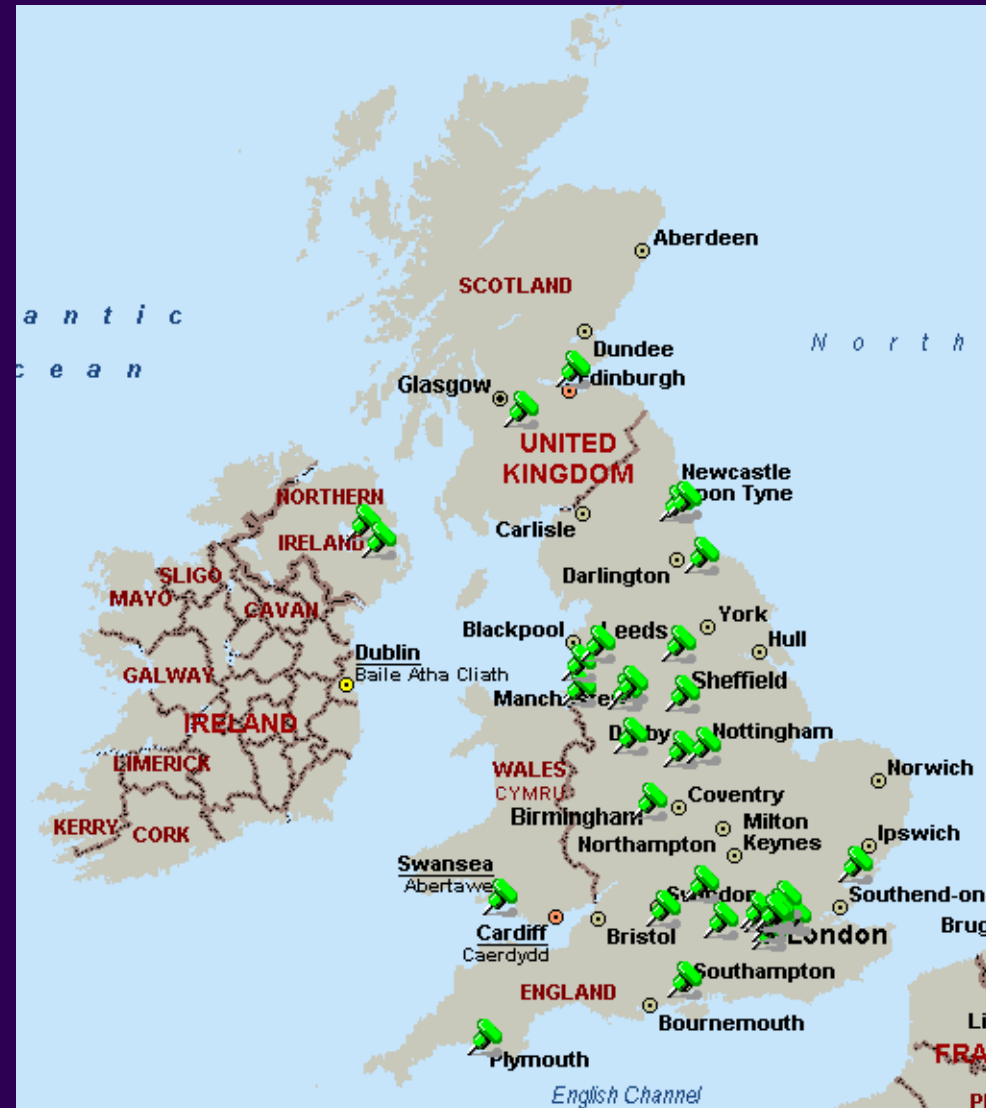
# OXA-48-like enzymes in the UK (Jan '12)



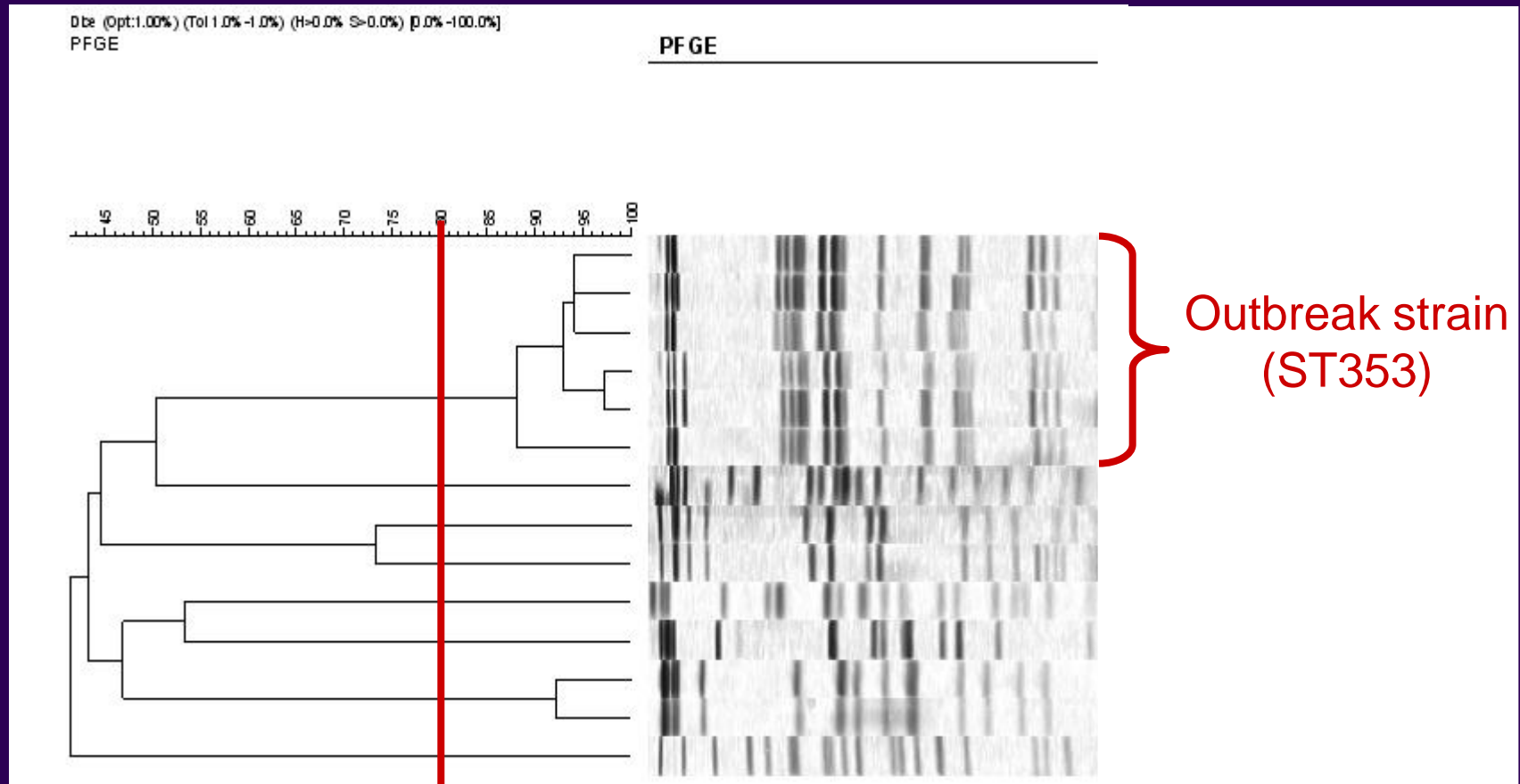
- 92 x Enterobacteriaceae

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

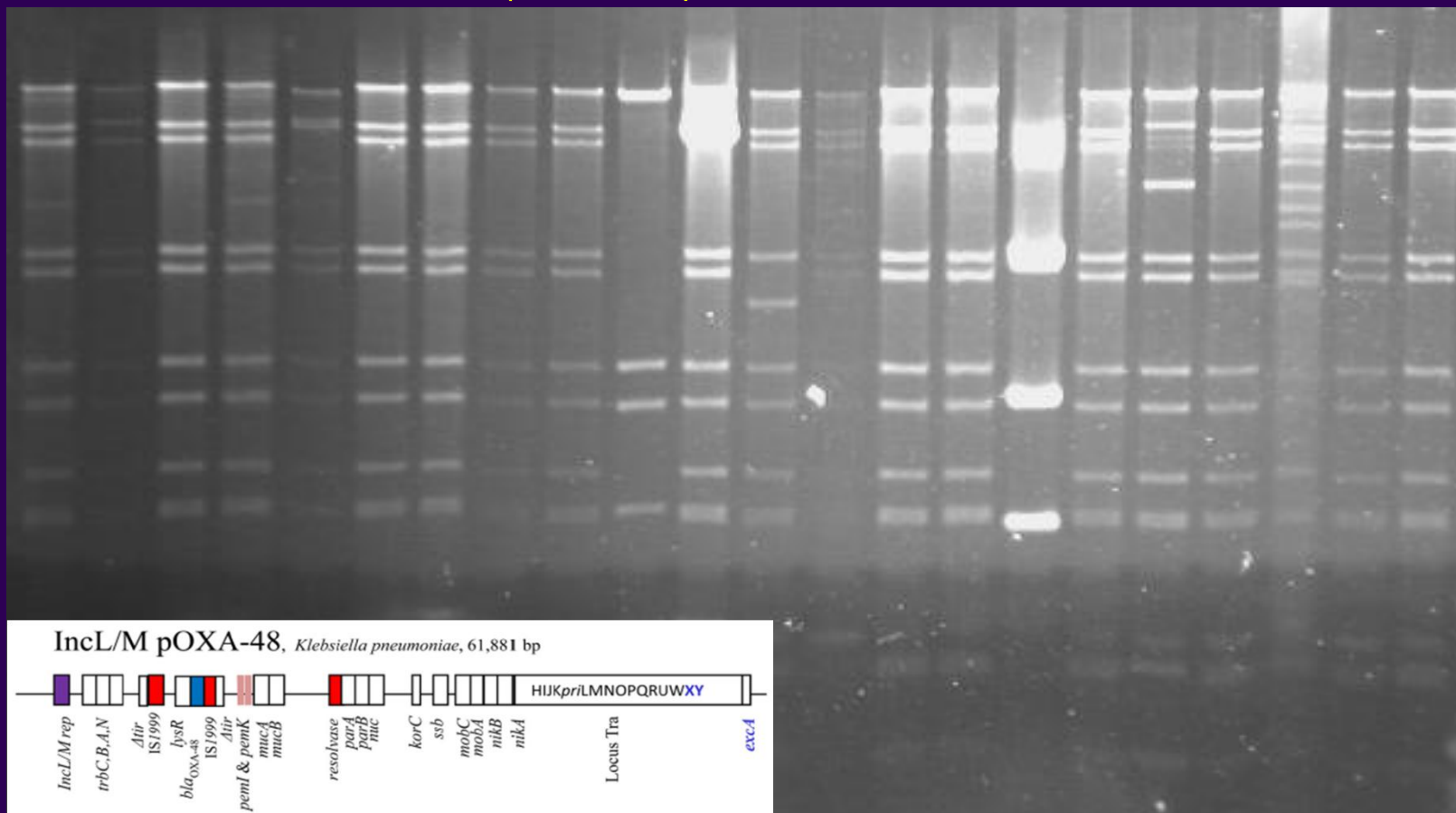
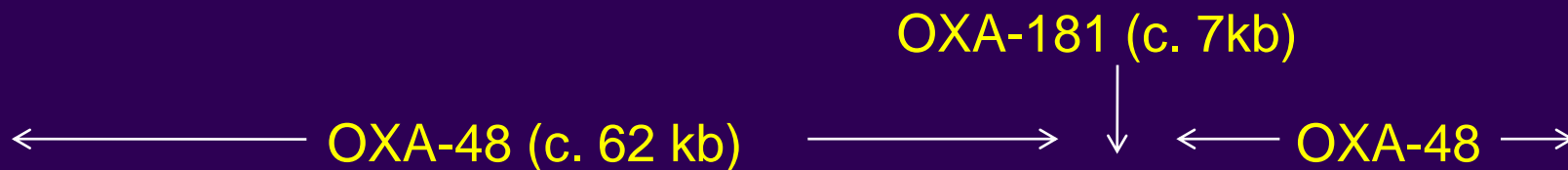
**Spread of highly related plasmids**



# OXA-48-producing *K. pneumoniae* in the UK



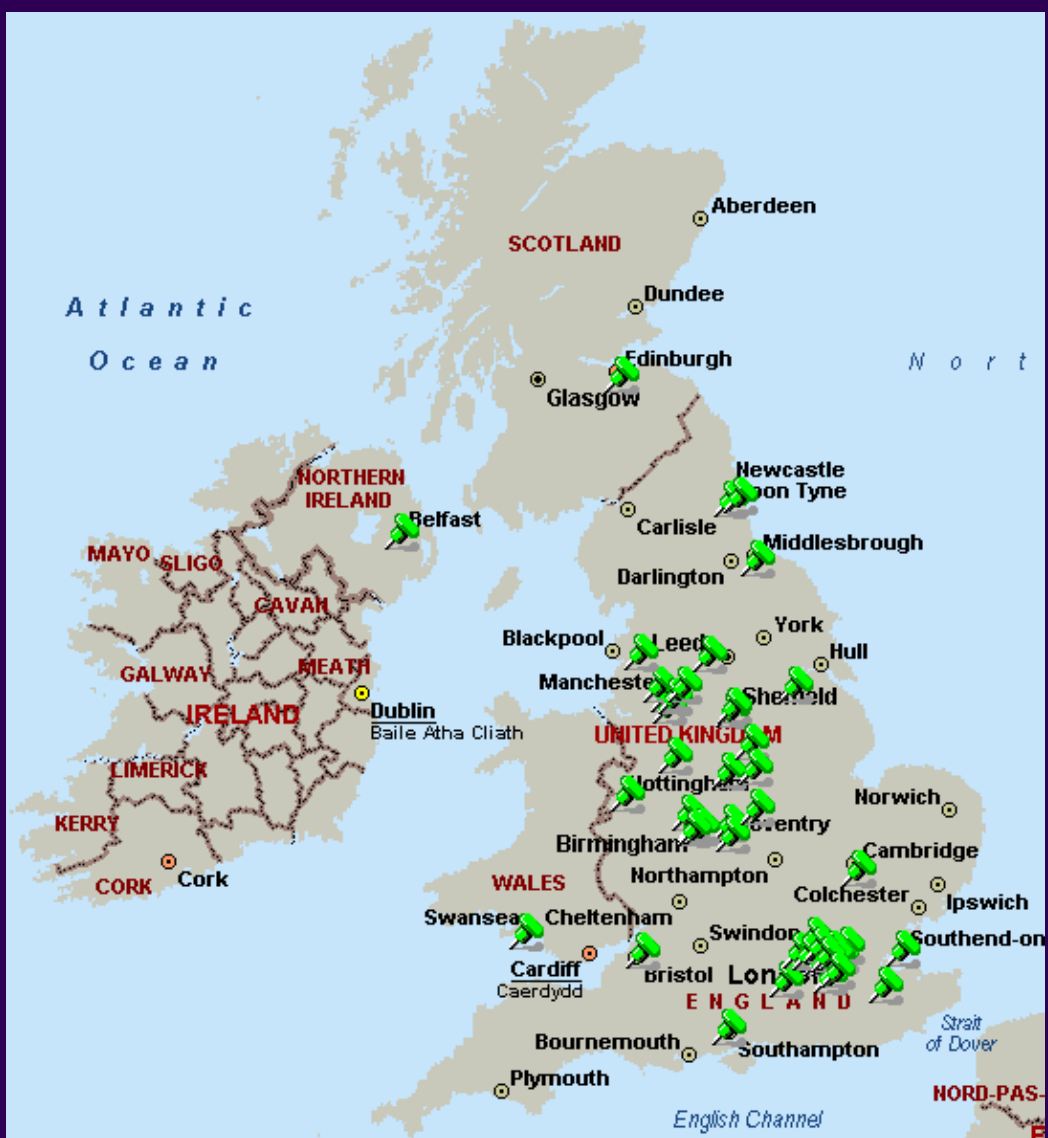
# International plasmid 'epidemic': OXA-48 plasmids in *Klebsiella*, *Enterobacter* and *E. coli*



# 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**





# $bla_{NDM}$ is on diverse plasmids

- IncA/C >> IncL/M, IncN, IncFI/II or NT

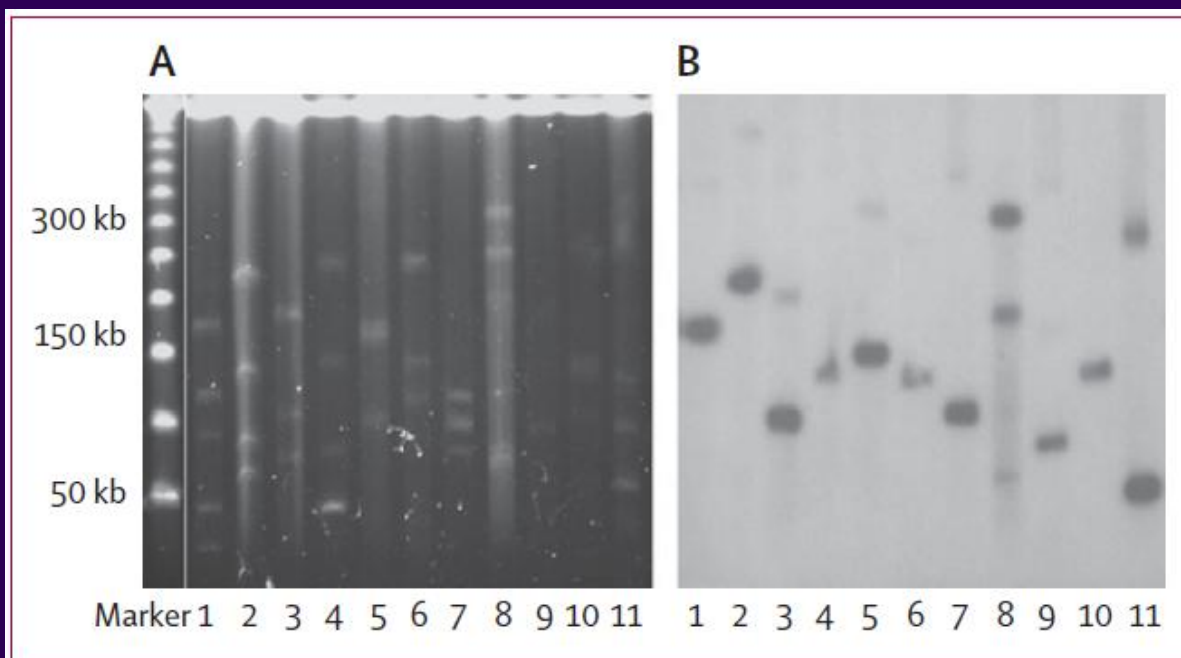


Figure 4: Hybridisation results of UK isolates with  $bla_{NDM-1}$

- Variation can be introduced during transfer / cloning

# ARMRL in national and European surveillance



- ARMRL undertakes sentinel surveys of resistance
- BSAC surveillance is sub-contracted to ARMRL
- ARMRL is partner in GRASP (Gonococcal Resistance to Antibiotics Surveillance Programme)
- ARMRL works in close relationship with *Salmonella* Reference Unit in HPA – Colindale
- ARMRL is major partner of EARSS

# Bacteraemia as a mandatory national surveillance



- 2173 *E. coli* bacteraemia isolates from 2001-2010 from 18 centres across UK and Ireland
- Phylotyping based on the Clermont et al. typing scheme (B2 most common group found)
- MLST using the Achtman scheme
- PFGE, virulence array, whole genome sequencing will be done on a subset of isolates

# Which *E. coli* clones cause bacteraemia (and UTI)?



ST	Bacteraemia 2001 (n=152)	Bacteraemia 2010 (n=116)		UTIs (n=300)*
73	20.5%	21.2%		16.6%
95	7.9%	11.0%		6.3%
131	<b>3.3%</b>	<b>13.6%</b>		12.3%
12	5.3%	7.6%		0.7%
127	4.6%	5.9%		3.6%
69	4.0%	5.1%		9.0%
Other	54.4% (58 STs)	35.6% (30 STs)		51.5% (97 STs)

- six major types increased from 45.6% to 64.4%, 2001 - 2010
- ST131 - most significant increase
- ST73 and ST95 not in ESBL +ve strain sets ...why ?

- Investigation of resistance to 'antibiotics of last resort' *i.e.* to drugs that usually remain active against bacteria resistant to other agents.

For gram-positive bacteria: oxazolidinones (linezolid), lipopeptides (daptomycin) and glycylyccline (tigecycline).

For gram-negative bacteria: imipenem, meropenem, and ertapenem, and the glycylyccline, tigecycline.

- ARMRL's research interests are focused on :

mechanisms of non-carbapenemase-mediated resistance to carbapenems in Enterobacteriaceae, particularly in *Klebsiella* spp. and *Enterobacter* spp.

mechanisms of resistance or reduced susceptibility to tigecycline in Enterobacteriaceae and *Acinetobacter* spp.

- the application of proteomics techniques to investigate novel and complex resistance phenotypes.

- Evaluation of new antibacterials in collaboration with the pharmaceutical companies.

Thank you...



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