Detecting carbapenemases in Enterobacteriaceae

David Livermore

Health Protection Agency,

Colindale, London

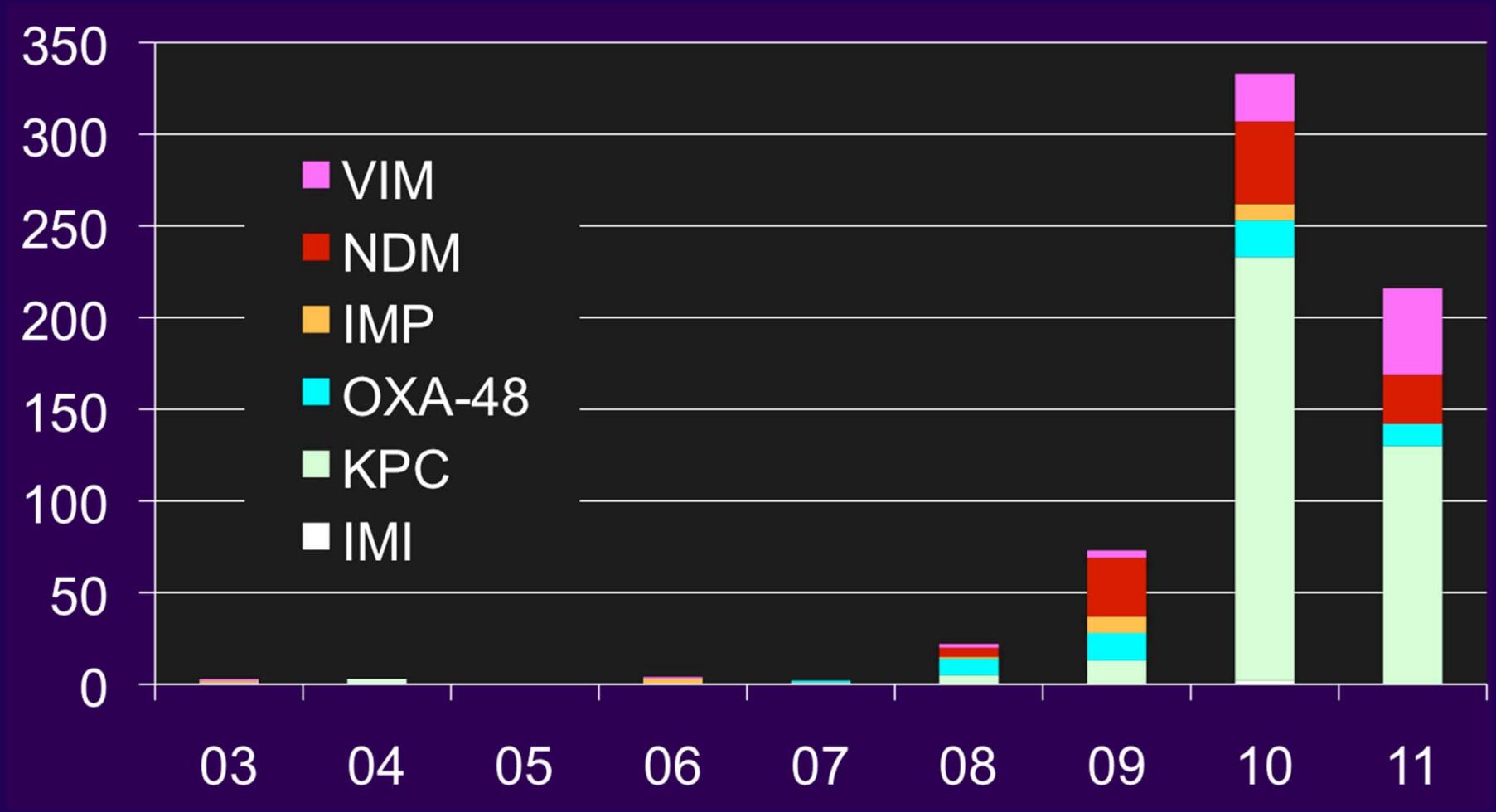
Mechanisms of carbapenem R in Enterobacteria

- Impermeability + AmpC or ESBL
- Metallo carbapenemases, IMP, VIM, NDM
- Non-metallo carbapenemases, KPC, OXA-48
- Carbapenemases the bigger risk, because:
  - Imperm + AmpC/ESBL strains unfit & unstable
  - Carbapenemase genes can spread

Why carbapenemases are harder than ESBLs

- Diverse types / inhibition profiles
- Resistance often low level
- Inhibitors used are less specific than clavulanate

Carbapenemase +ve Enterobacteria referred to ARMRL, 2003-May 2011



HPR June 17th 2001

When to be suspicious of a carbapenemase

- **Test ertapenem & meropenem (or imipenem)**
- **Suspect any isolate with reduced S to either drug**

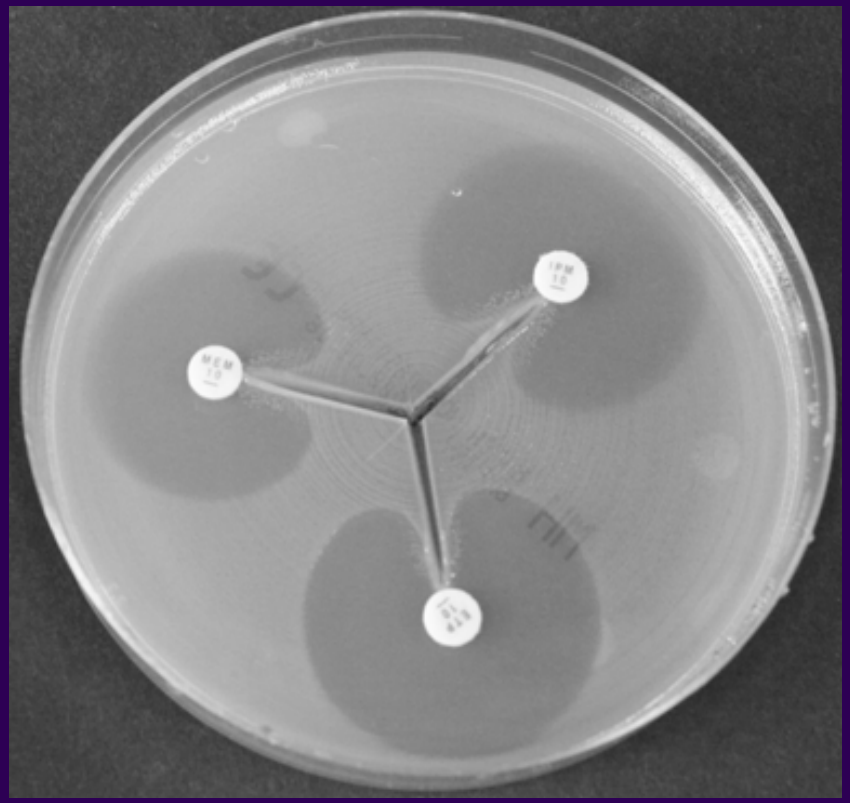
EXCEPT

- Proteeae I/R to imipenem only
  - Intrinsic trait of the genus, esp. *P. mirabilis*
- Enterobacter with I/R to ertapenem only and R to cephs
  - Almost certainly derepressed AmpC + impermeability

When is impermeability + AmpC/ESBL more likely?

- All carbapenem MICs <2 mg/L
- MIC ertapenem >> MIC imipenem / meropenem
  - e.g. ertapenem 8, imipenem 0.5, meropenem 0.12 mg/L
- When other synergies suggest ESBL / AmpC
  - But ceph/clav MICs not quite as low as normal
  - e.g. cefotaxime >256, cefotaxime+clav 2 mg/L, not 0.06
- When fully S to non-β-lactams

Clover leaf (Hodge) test for carbapenemase



Test strain

K. pneumoniae

KPC

Indicator

E. coli NCTC10418

Discs

IMP, MEM, ERT 10 μg

Cumbersome & imperfect…. But still useful

false +ve with AmpC + impermeability, not ESBL

Synergy tests for carbapenemases

	MBLs	KPC	OXA-48
EDTA	+	-	-
Dipicolinic acid	+	-	-
Boronic acids	-	+	-
Clavulanate	-	Weak	-
(NXL104)	-	+	+

Formats for synergy tests

- Double disc (Rosco sell dipicolinic acid & boronic acid discs – use combined with meropenem)
- Combination discs – supplement carbapenem discs with EDTA and compare zones
- Double-ended Etests
- Agar dilution: imipenem +/- e.g. EDTA at 320 mg/L
- Mostly validated on MH agar, not IsoSensitest

Etest for metallo-β-lactamase

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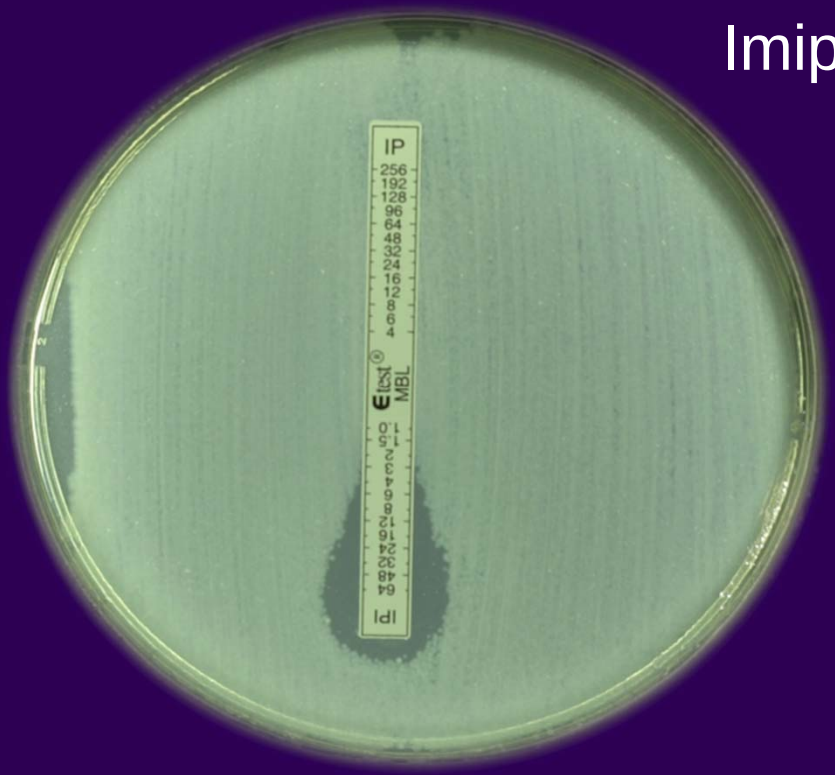
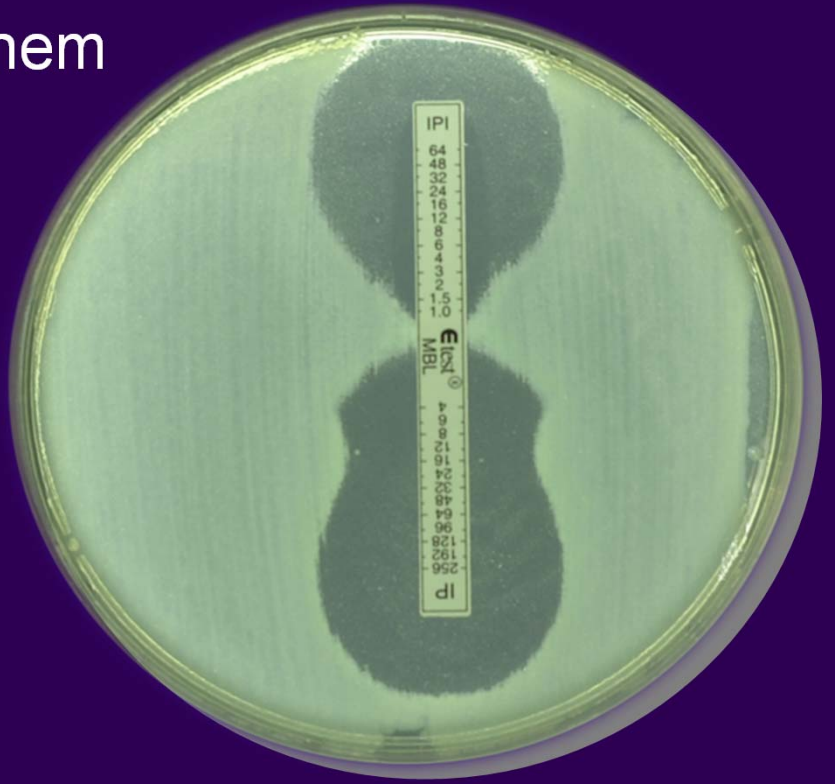
Imipenem



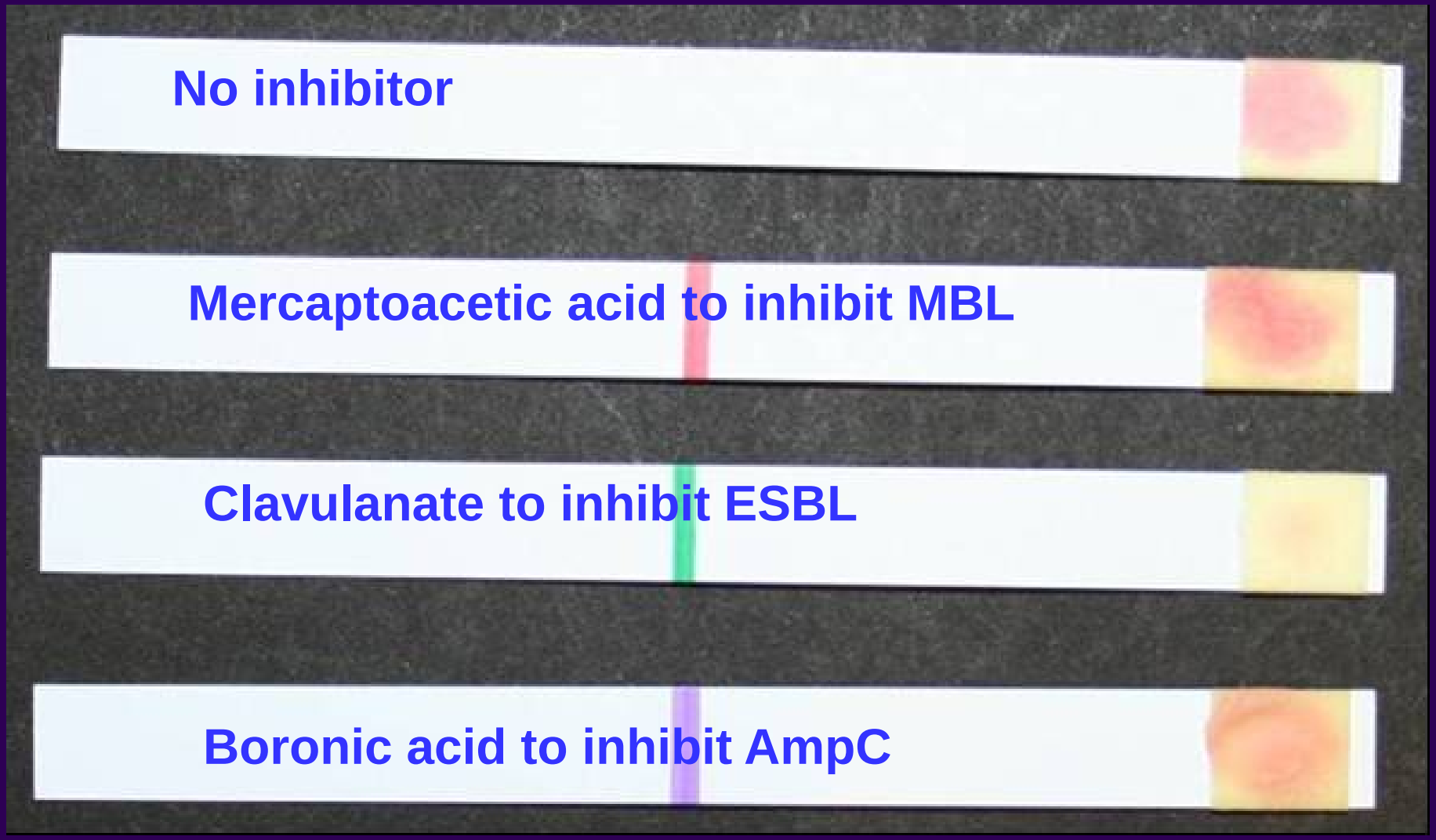


Imipenem+ EDTA

Cica β-Test (Mast)

- Examine hydrolysis of chromogenic oxyimino ceph, HMRZ-86- yellow to red
- If +ve, test inhibition IN SEQUENCE by:
  - Sodium mercaptoacetic acid – MBL
  - Clavulanic acid – Class A / ESBL
  - Benzo-thiophene-2-boronic acid – AmpC
- Count first positive result

Cica β-Test (Mast)



Cica β-Test (Mast) blind testing of overnight cultures

	Mechanism inferred					
Reference data	**MBL**	**ESBL**	**AmpC**	**Mixed Other**	**Pen' ase**	**No activity**
MBL (26)	**20**	1	0	2	3	0
ESBL (74)	3	**63**	2	6	0	0
AmpC (25)	2	0	**18**	3	2	0
K. oxytoca, K1 (10)	0	2	6	**2**	0	0
OXA carbapenemases (10)	0	0	0	**10**	0	0
P. aeruginosa OXA ESBLs (4)	1	3	0	**0**	0	0
KPC/SME carbapenemase (2)	0	0	2	**0**	0	0
Penicillinase (39)	5	3	1	0	**30**	0

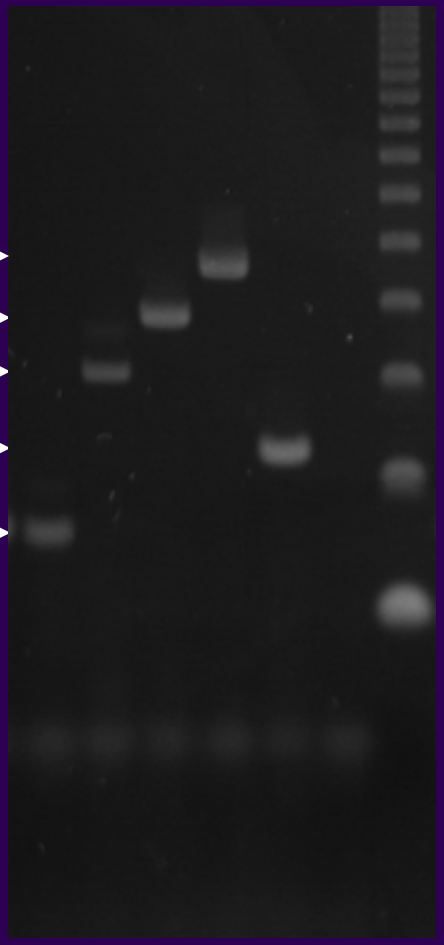
Better but slower to use with antibiogram @ 48h

Livermore et al., JAC in press.

Identifying carbapenemases at ARMRL

- If EDTA test +ve
  - Multiplex for IMP, VIM, GIM, SPM
  - Single PCR for NDM
  - NDM first if relevant travel & R all aminoglycosides
- If EDTA test –ve
  - Single PCRs for OXA-48 and KPC; IMI & Sme if -ve

Multiplex detection of MBL carbapenemase genes



6 different *bla*$_{IMP}$ alleles

IMP-1, -2, -4, -7, 12, -13

6 different *bla*$_{VIM}$ alleles

VIM-1, -2, -4, -7, -9, -10

Referred isolates, UK

64 VIM producers

15 IMP producers

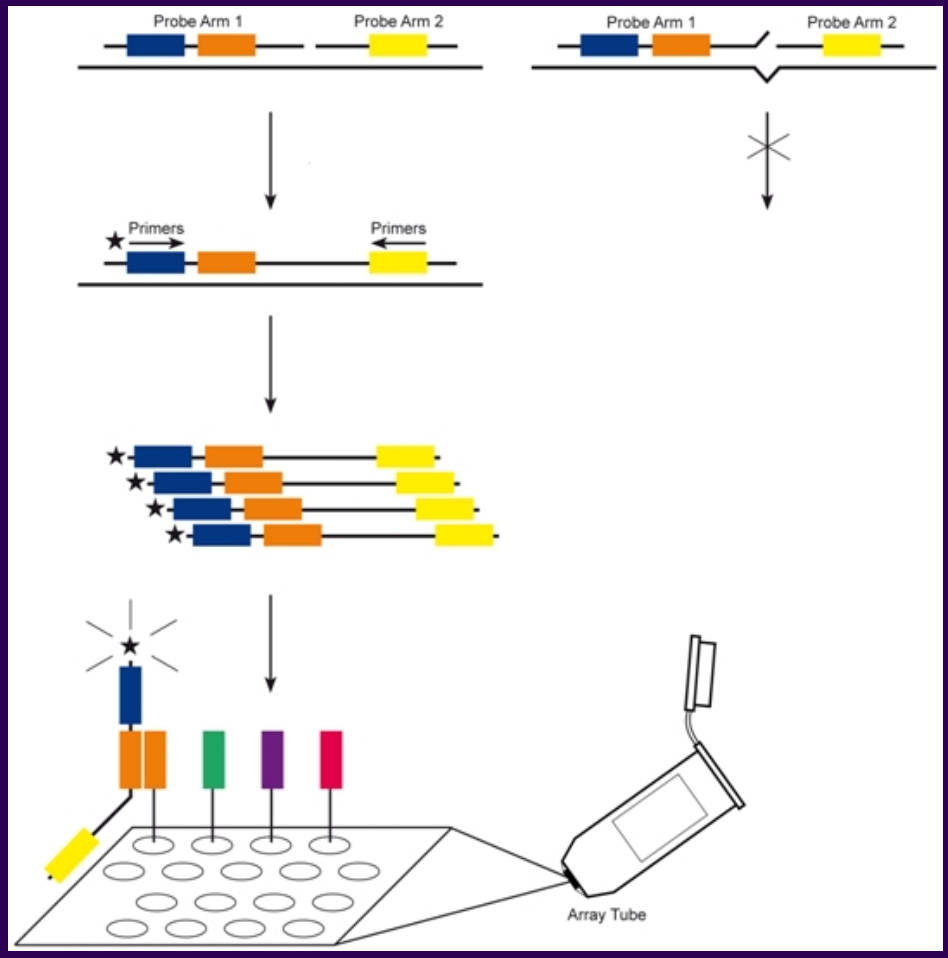
Ellington *et al*. *JAC* 2007*;* **59:** 321

Also;

TaqMan PCR for KPC, IMI/NMC, SME, GES & OXA-48

Swayne *et al., IJAA* 2011; **38:** 35

Checkpoints array for carbapenemases, ESBLs & AmpC



	Array +ve	Array -ve
OXA-48 (11)	11	0
KPC (8)	8	0
IMP (12)	12	0
VIM (3)	3	0
NDM (7)	7	0
Imperm (16)	0	16
-ve Controls (7)	1	6

http://www.check-points.com

Test time

	Kleb 1	Ent'bacter	Kleb 2	Kleb 3	Kleb 4
Cefotaxime	32	256	128	1	64
+clav	4	128	64	1	16
+cloxacillin	32	8	128	1	32
Ceftazidime	16	128	128	2	32
Cefepime	16	16	64	1	16
Aztreonam	32	128	64	4	0.25
Ertapenem	8	8	32	8	4
Meropenem	4	1	32	4	2
Imipenem	8	2	64	2	4
+EDTA	4	1	0.5	4	0.25
Gent/Tob/Amik	KPC	Imperm	NDM	OXA-48	VIM/IMP

Summary

- Carbapenemases present a diverse threat
- Be suspicious, even of low level resistance
- Look at the patient's history
- Look at complete resistance patterns, not just 'indicators'
- Refer any meropenem R Enterobacteriaceae
- No need to refer Enterobacter if Erta-R, Mero-S
- Where HPA-ARMRL confirm, PLEASE complete the patient record form; but don't use this to refer the original isolate!