

# ESBLs and MBLs

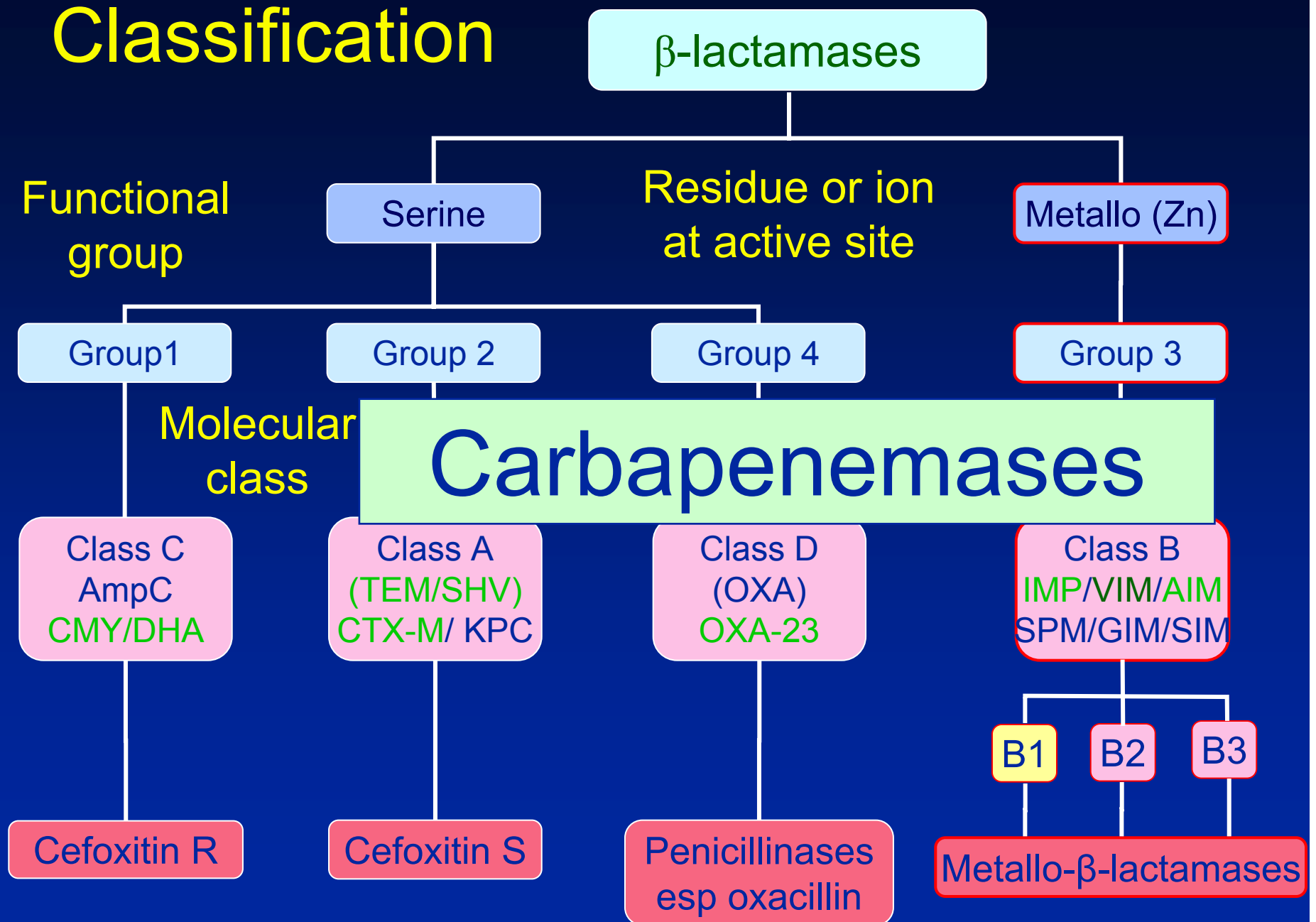
## ESBLs and Carbapenemases



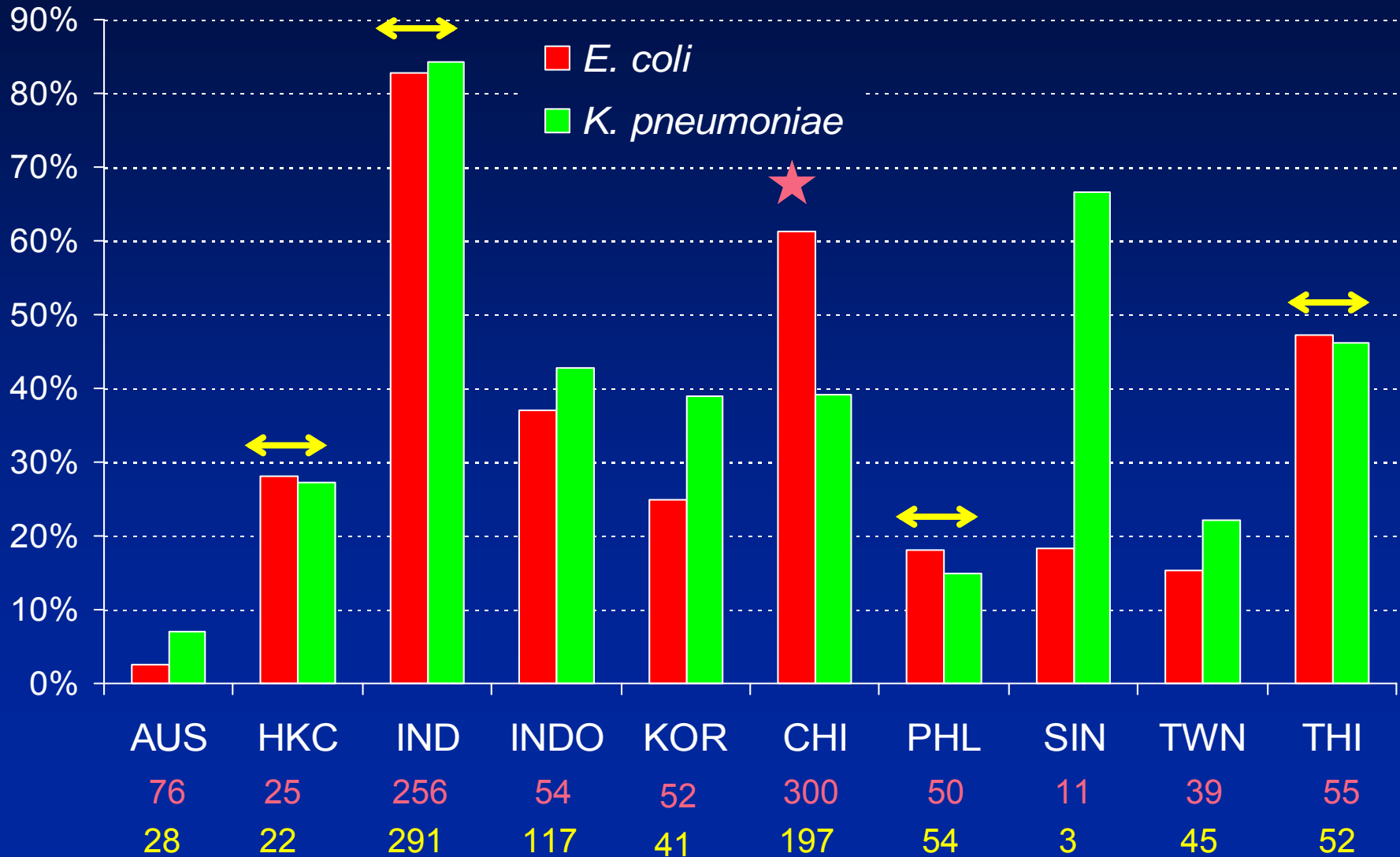
# Outline

- Terminology
- Prevalence
  - Global and local
- Detection Issues
  - Extended-spectrum  $\beta$ -lactamases
  - Metallo- $\beta$ -lactamases
- Relevance to a lab near you!

# Classification



# Extended-spectrum- $\beta$ -lactamases 2006



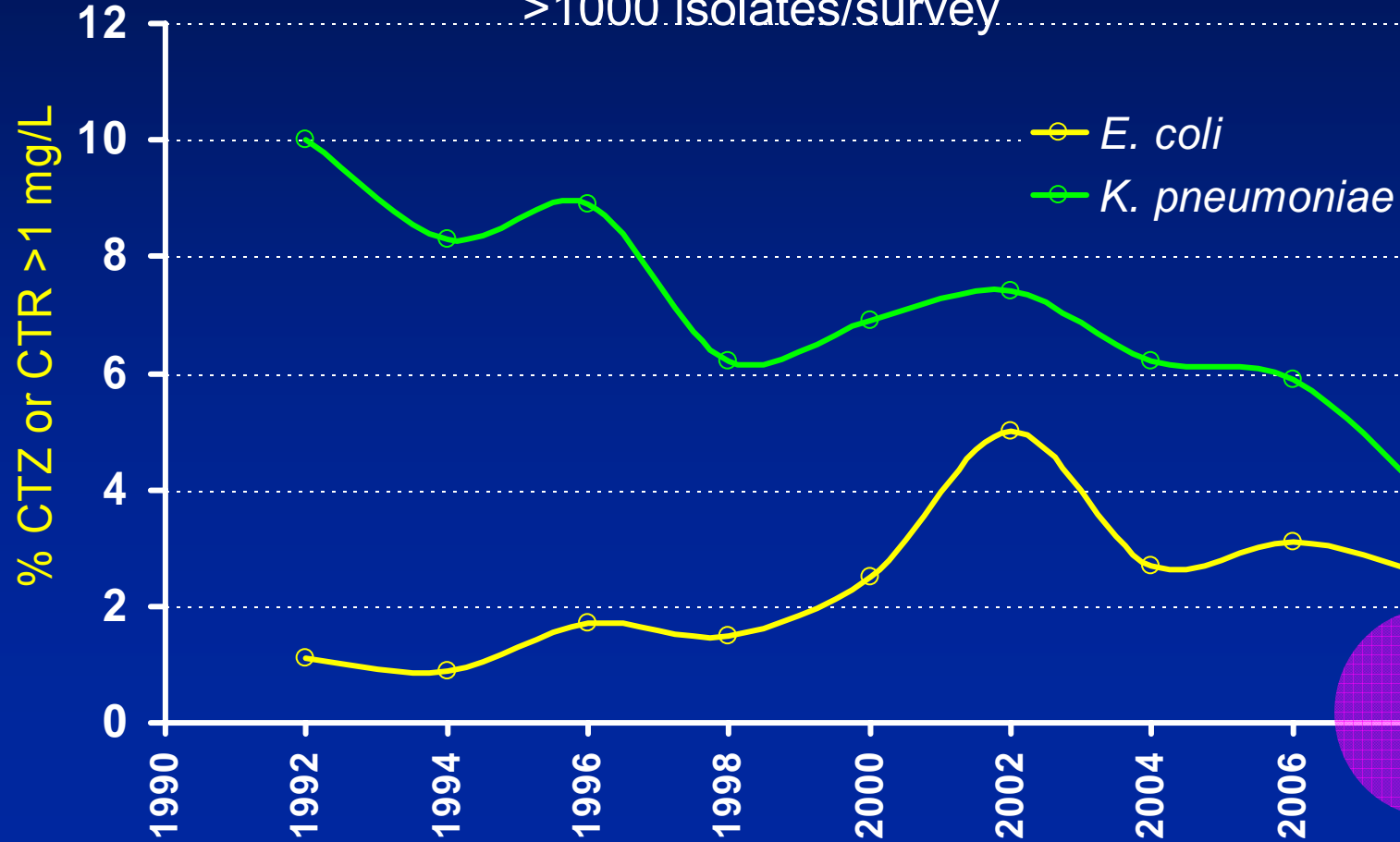


## *Escherichia coli* and *Klebsiella pneumoniae*

### ESBL phenotype

Hospital + Community-onset

>1000 isolates/survey



# Extended-Spectrum $\beta$ -Lactamases

- TEM variants
  - all variants of TEM-1
    - origin unknown (patient named Τεμονειρα )
- • SHV variants
  - all variants of SHV
    - the natural *K. pneumoniae* chromosomal  $\beta$ -lactamase
- • CTX-M variants
  - 4 separate groups
    - Groups 1, 2, 8/25 and 9, all from *Kluyvera* species
- OXY variants
  - From *Klebsiella oxyoca*
    - natural enzyme but ESBL activity when hyperproduced

# Plasmid-borne AmpC-like Enzymes

## 6 Families

- Characterised by ability to hydrolyse cephamycins, e.g. cefoxitin, as well as 3<sup>rd</sup> generation cephalosporins

- **C-1 (CIT) family** [CMY-2 to 7, LAT-1,3,4]

- from *Citrobacter freundii*

- **C-2 (MOX) family** [MOX-1 to 2, FOX-1 to 5, CMY-1, 8-9]

- from *Aeromonas hydrophila* and *sobria*

- **C-3 (ACT) family**

- from *Enterobacter asburiae*

- **C-4 (DHA) family**

- from *Morganella morganii*

- **C-5 (ACC) family**

- from *Hafnia alvei*

- **C-6 (EBC) family** [MIR-1, ACT-1]

- from *Enterobacter cloacae*

**CIT = From *C. freundii***

**MOX = Active against moxalactam**

**ACT = AmpC-type**

**DHA = From Dhahran, Saudi Arabia**

**ACC = Ambler class C**

**EBC = From *E. cloacae***

# B-lactamases

Plasmid encoded

- 1983 SHV-type (> 120)
- 1985 TEM-type (> 160)
- 1989 CTX-M-type (> 90)



“classical ESBLs”

ESBLs of growing importance

- 1988 SFO-1 *Serratia FOnticola*
- 1991 TLA-1 *TLAhuicas* (indian tribe)
- 1991 PER (6) *Pseudomonas Extended Resistance*
- 1996 VEB (7) *Vietnam Extended-spectrum β-lactamase*
- 1996 BES-1 *Brazilian Extended-Spectrum β-lactamase*
- 1998 GES (12) *Guyana Extended-Spectrum β-lactamases*
- 2005 BEL-1 *Belgium Extended-spectrum β-Lactamase*
- 2005 TLA-2 ???? (Plasmid, waste water)



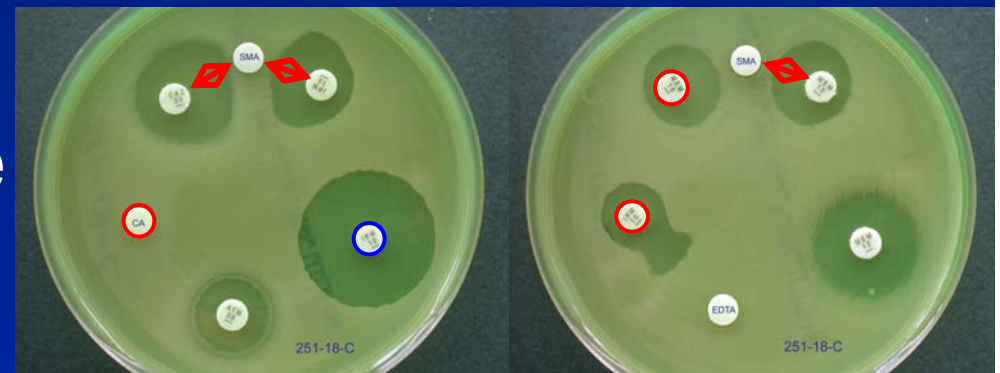
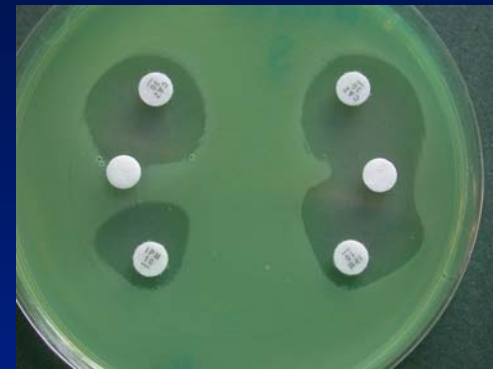
“Infrequent ESBLs”

- 1998 KPC (10) *Klebsiella pneumoniae Carbapenemase*
- 1991 OXA-ESBL (OXA-1, OXA-2 and OXA-10-types)

# Carbapenem-resistance

## Key Organisms

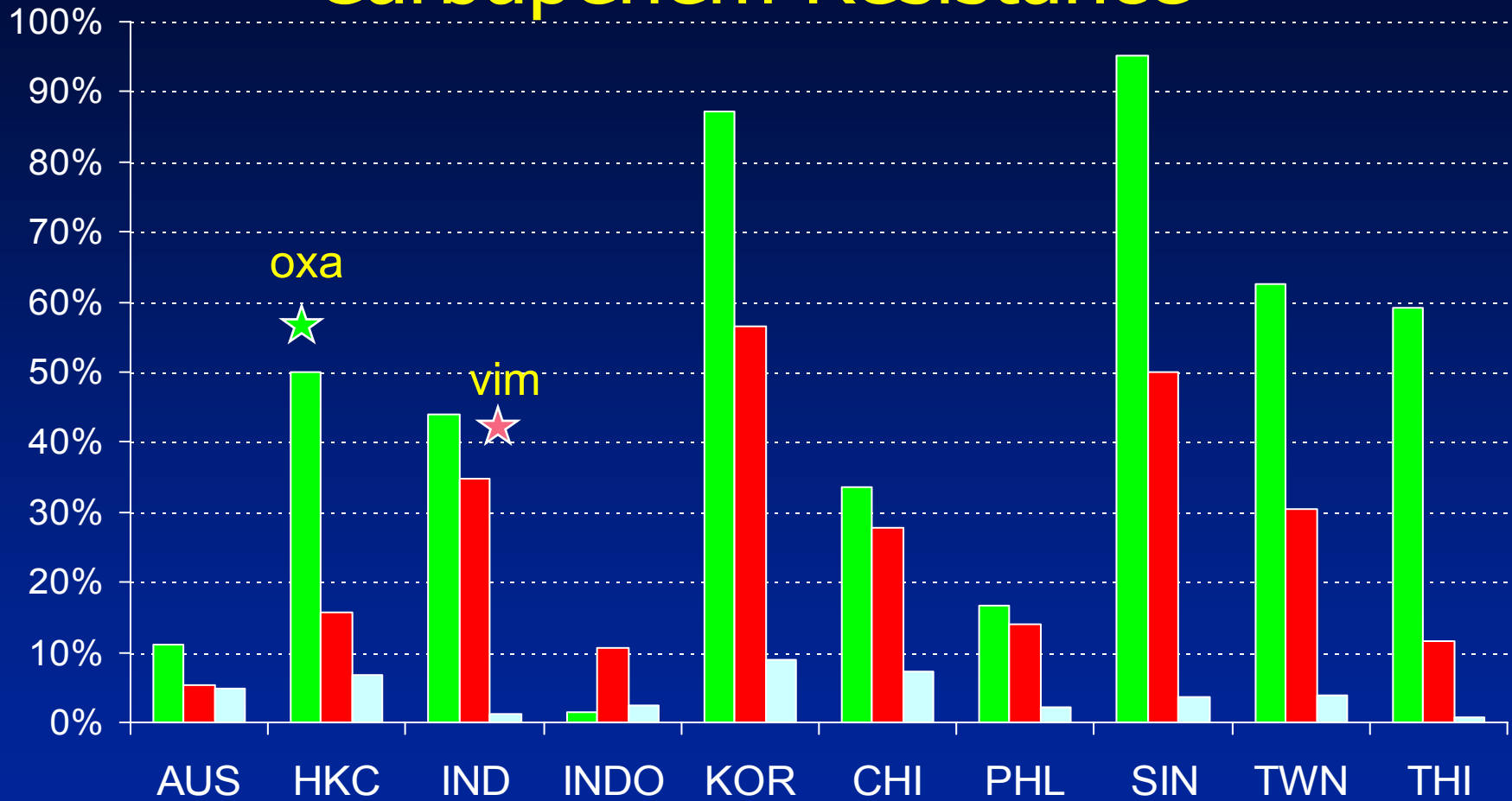
- *Pseudomonas* spp.
  - metallo- $\beta$ -lactamases
- *Acinetobacter* spp.
  - Class D
  - Class B
- Enterobacteriaceae
  - Class A
  - Class B



**Rapidly emerging problem!**

2006

# Carbapenem Resistance



■ *Acinetobacter* (n=546)

■ *Pseudomonas* (n=752)

■ *Enterobacteriaceae* (n=2,474)

Imipenem/meropenem  $\geq 8$  mg/L

Class B/D

Class B

$\geq 2$  mg/L

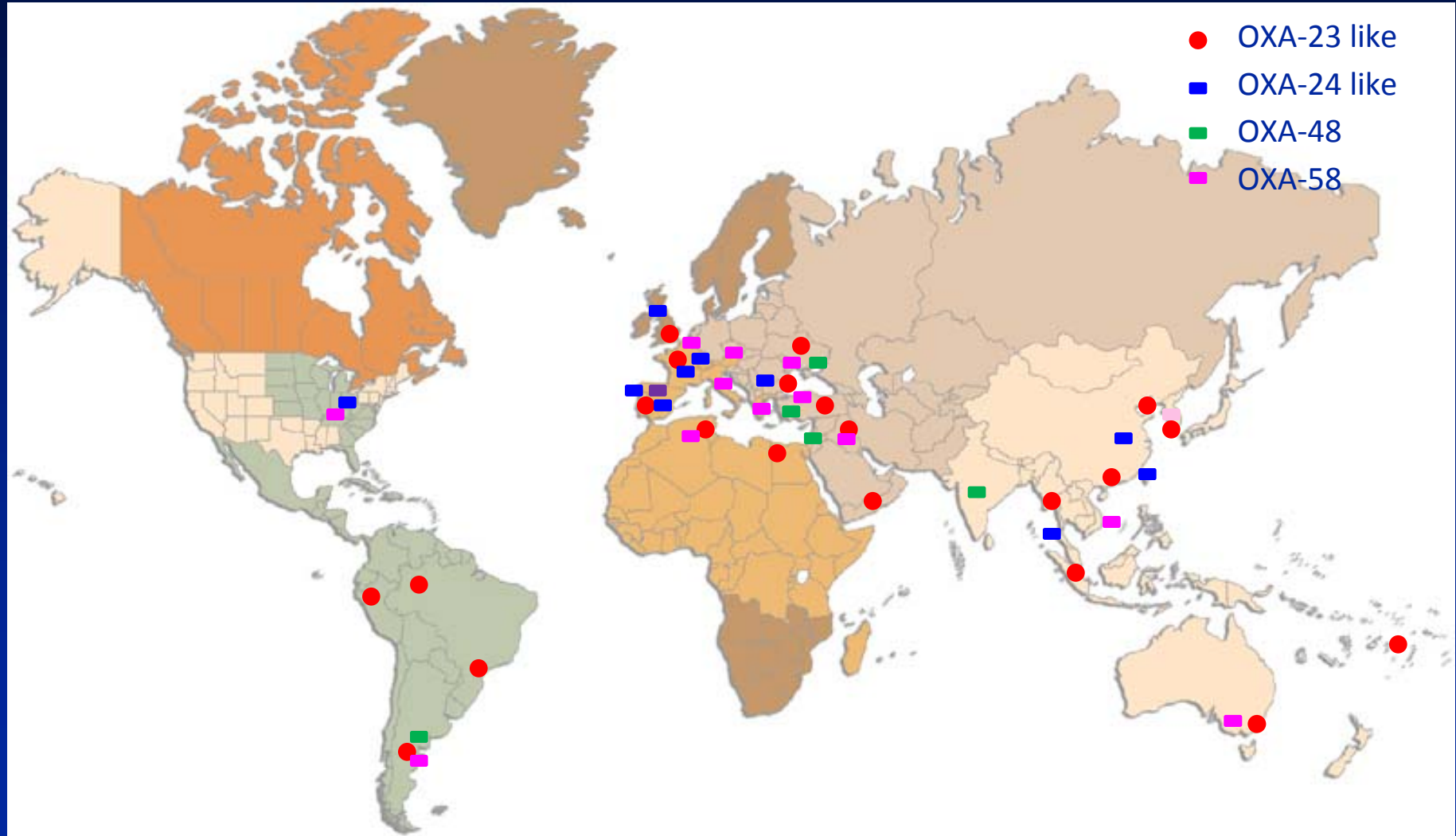
Class A/B

# OXA- Carbapenemases

- OXA variants

- Some OXA variants are “narrow spectrum”
- Some are ESBLs
- Important ones are almost exclusive to *Acinetobacter*
- *A. baumannii* contains OXA-51 naturally
- 9 subgroups so far described
- ➤ Most important for us are OXA-23 and OXA-58
- OXA-48 (*Klebsiella pneumoniae*) coming

# Global Distribution of OXA-type Carbapenemases



# Metallo- $\beta$ -lactamases

- **VIM** variants

- 23 described so far
- Mostly found in *P. aeruginosa* but also in *Acinetobacter* spp. in some countries
- Very common in Greece, India and Korea, where they are also a problem in Enterobacteriaceae
- Occasionally introduced into Australia from these countries

- **IMP** variants

- 26 described so far
- Found in *P. aeruginosa* *Acinetobacter* spp. and Enterobacteriaceae
- Only endemic metallo- $\beta$ -lactamase in Australia (Melbourne, Sydney, Canberra) (IMP-4)

**VIM** = Verona integron-associated metallo- $\beta$ -lactamase

**IMP** = Active against imipenem

# Metallo- $\beta$ -lactamases

- Others

- **SPM** in Brazil
  - common in a range of Gram-negatives
- **GIM** and **SIM**
- **NDM** in India
  - Rapid spread, now in many Gram-negative species
- **KHM** in Japan
  - from *C. freundii*
- ➤ **AIM** in **Australia**
  - from *P. aeruginosa* & *P. putida*

**SIM** = Seoul imipenemase

**SPM** = São Paulo metallo- $\beta$ -lactamase

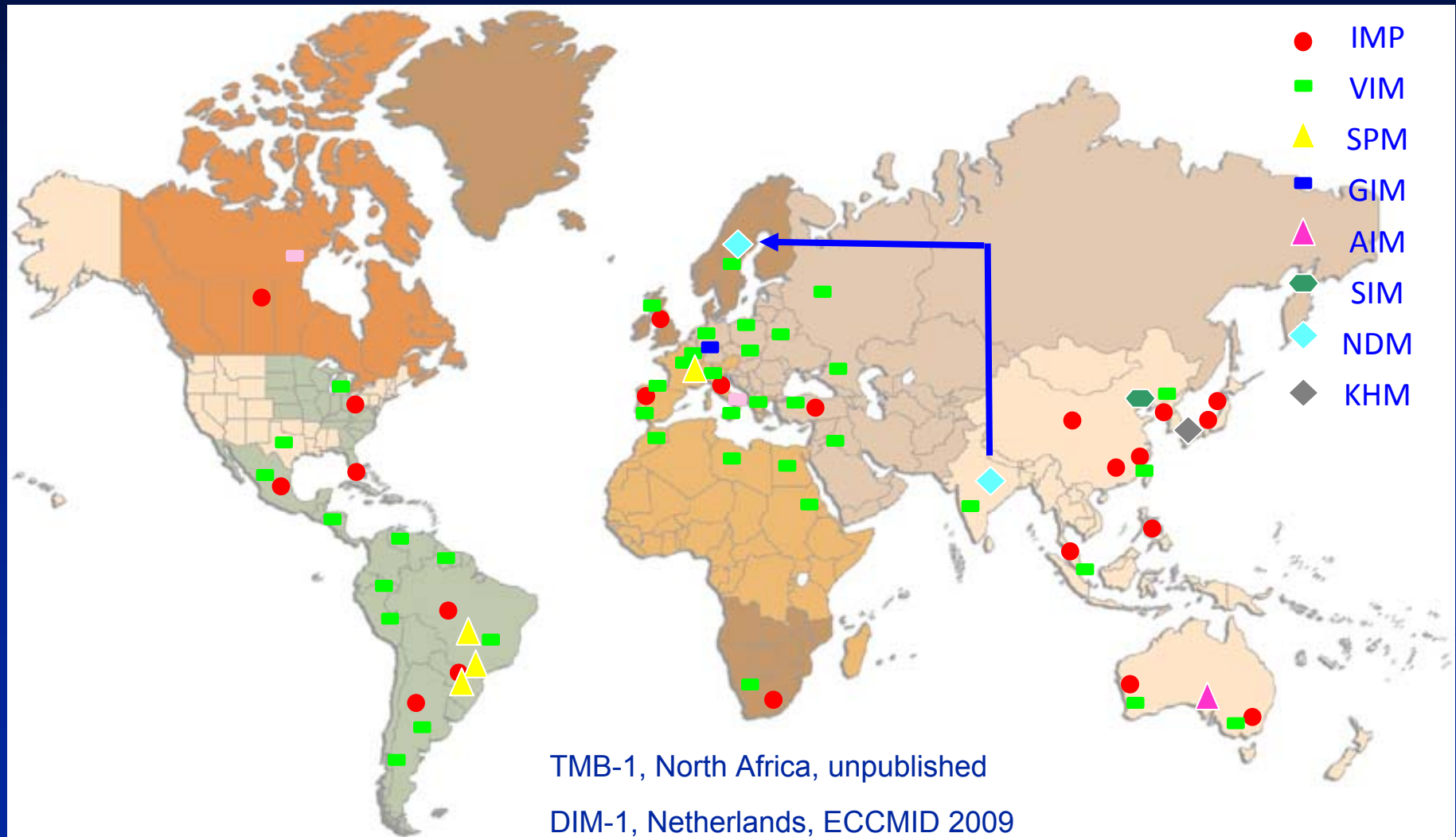
**GIM** = German imipenemase

**NDM** = New Delhi imipenemase

**AIM** = Australian imipenemase

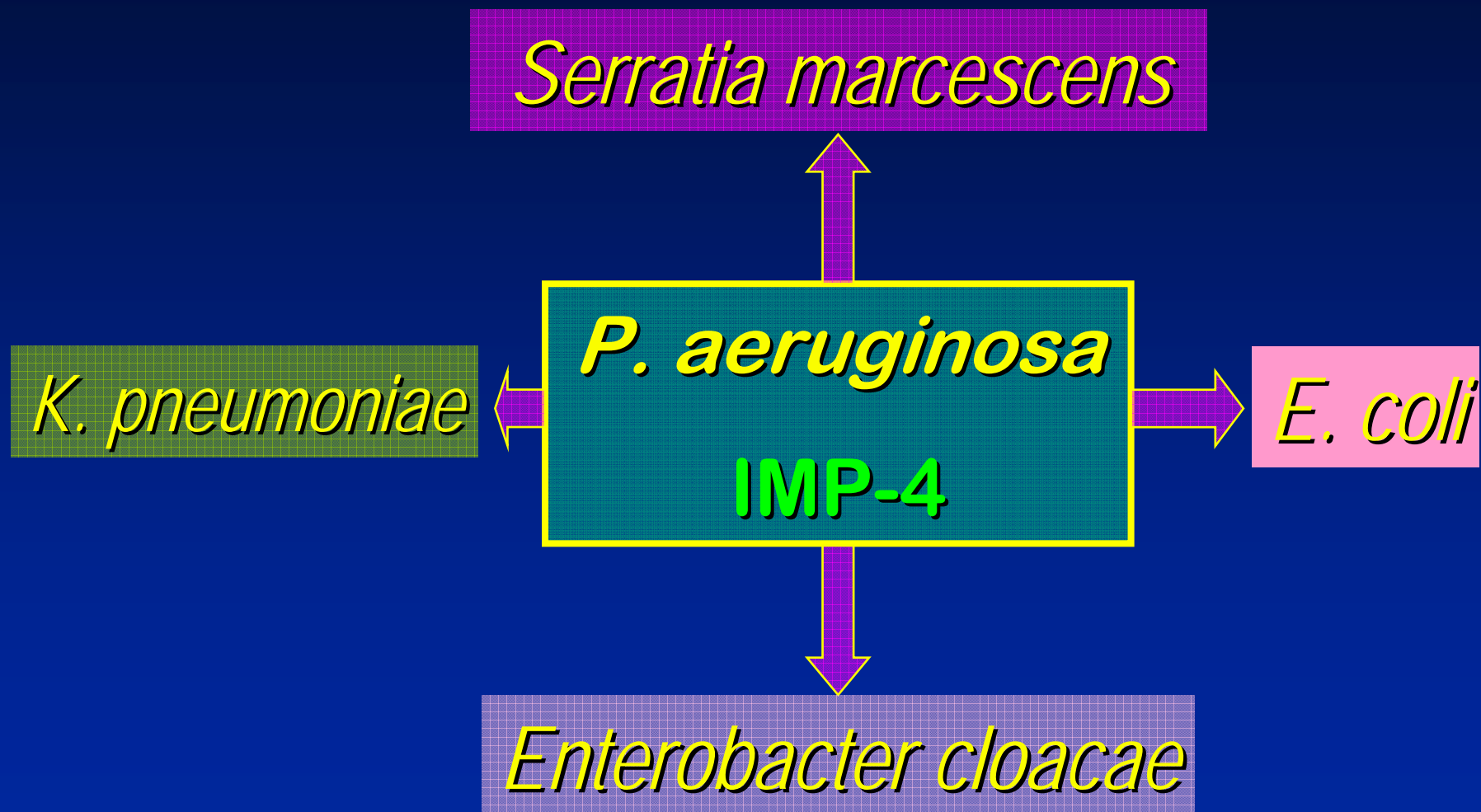
**KHM** = Kyorin University Hospital imipenemase

# Location of Mobile MBL- Containing Organisms

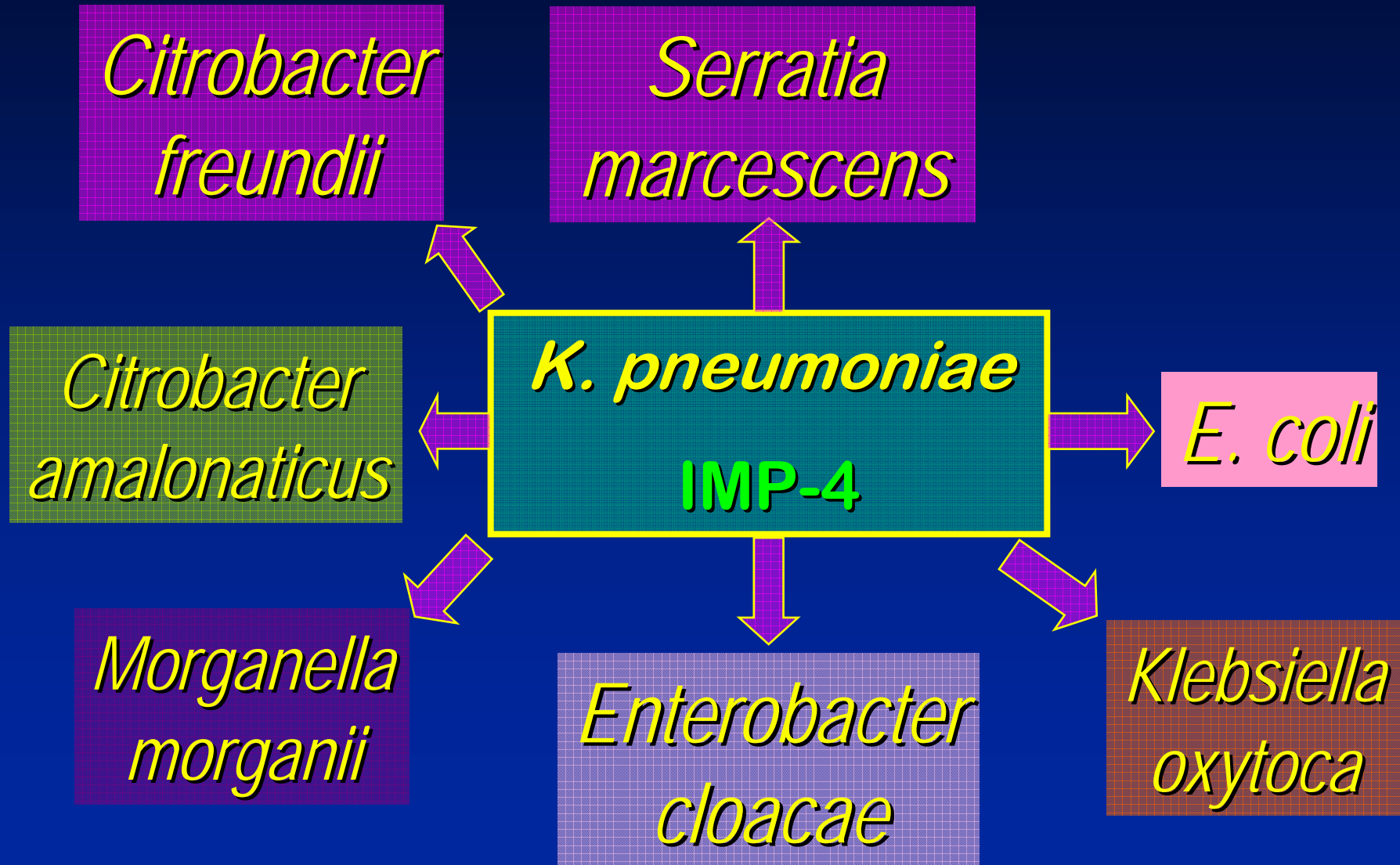




## A Home-Grown Problem 2004!



# Another Home-Grown Problem in Western Sydney



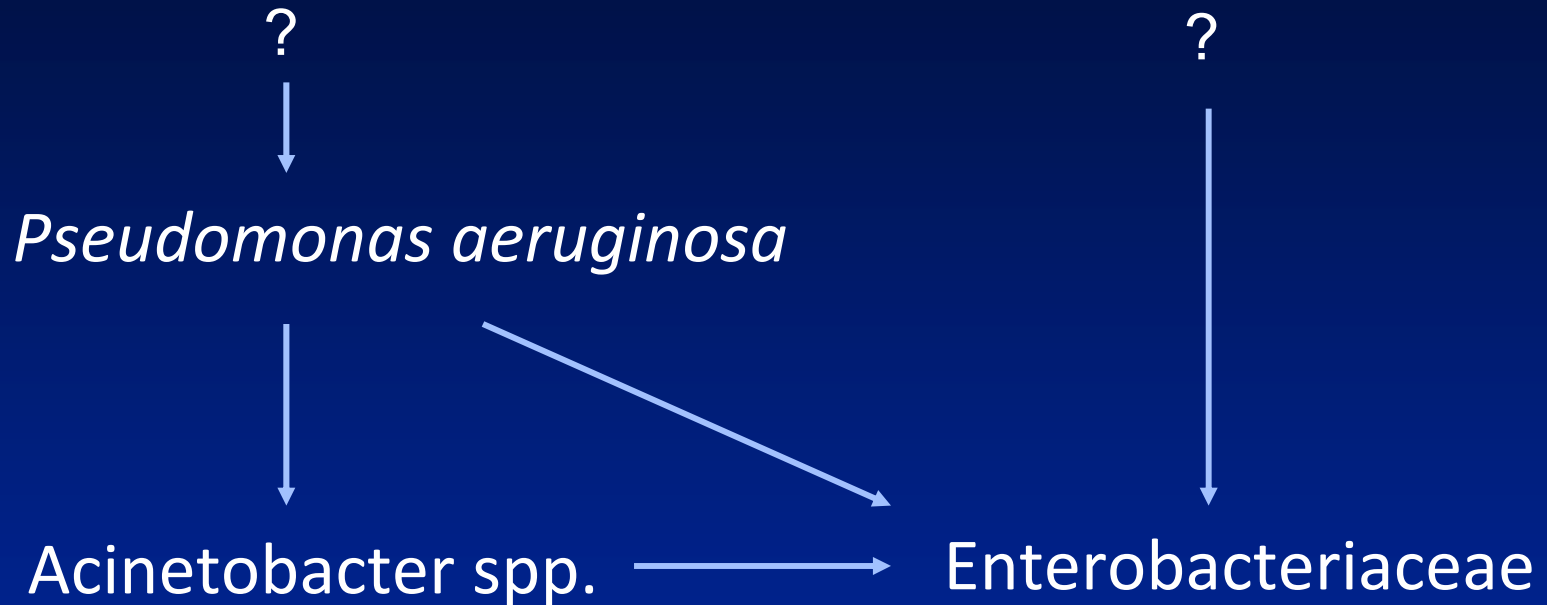
## AIM-1 in Adelaide

- Metallo-beta-lactamase
  - B3 subclass
  - Incorporated in an ISCR element
  - ? Environmental source
- 10 isolates/patients since 2006
  - *P. aeruginosa* x 8, and *P. putida* x 1
- First case transferred in from Alice Springs
- All isolates from RAH

# The Evolving Resistances in Gram-Negatives

|                              | <i>Enterobacteriaceae</i> | <i>P. aeruginosa</i> | <i>A. baumannii</i> |
|------------------------------|---------------------------|----------------------|---------------------|
| ESBLs                        | +++                       | +                    | +                   |
| Carbapenemases               | +                         | +++                  | ++                  |
| Aminoglycoside R             | ++                        | ++                   | +                   |
| Plasmid-borne<br>Quinolone R | ++                        | +                    | +                   |

## Where are MBLs found?



metallo-β-lactamases mimic ESBLs on  
many issues not least detection in  
Enterobacteriaceae

# Detection of $\beta$ -lactamases

- Phenotypic detection of  $\beta$  lactamases problematic
  - wide range of enzyme types
  - wide range of substrate specificities
  - variable inhibitory profiles
  - acquisition of multiple enzyme classes
  - ability to share/spread to other genera/species

# ESBL Detection Methods

- Screening tests
- Double disc synergy test (DDST, keyhole, **Jarlier**)  
spacing of discs, disc strength, type (CTX, CTR, CAZ, AZT)
- Replacement method (overlay, **Casals**)  
enhancement of zone diameters
- Combination discs
- Etest<sup>®</sup> ESBL
- Automated Systems

# Combination Disc Tests

- Overcome disc spacing issues
- Use clavulanic acid
- Commercially available
- Same conditions as routine AST method
- Measure zone diameters

Oxoid, BioRad, MAST, BBL

# ESBLs in the presence of AmpC $\beta$ -lactamases

## Disc based methods

- 3 dimensional test
- EDTA discs
- **Boronic acid** 3-aminophenylboronic (APB)

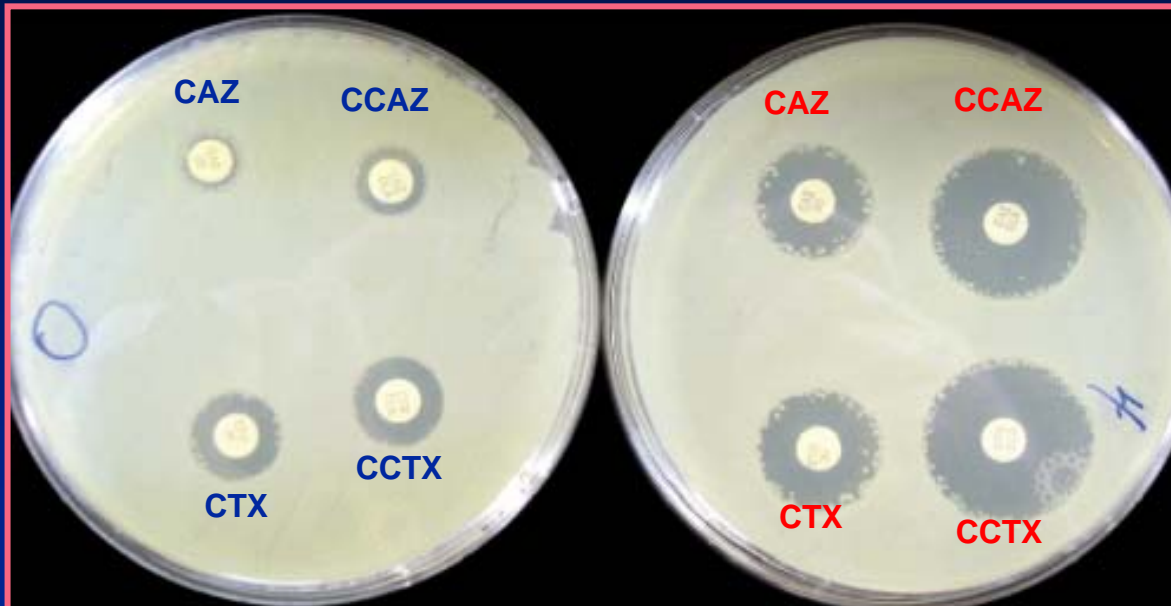
## Agar based methods

- Cloxacillin (200 mg/L)
  - cloxacillin inhibits cephalosporinase activity

# ESBLs in the presence of AmpC $\beta$ -lactamases

Without cloxacillin

With cloxacillin



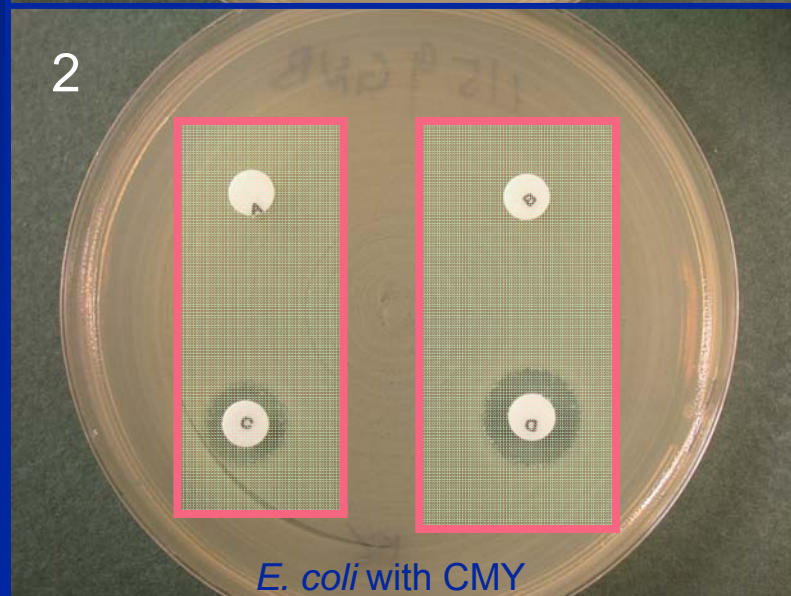
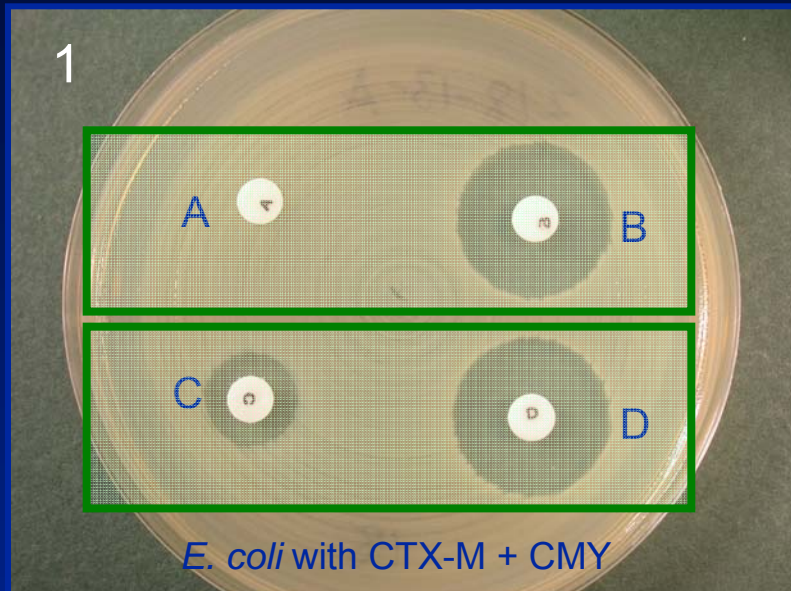
- AmpC  $\beta$ -lactamase inhibited without inhibiting ESBL-producing organisms

> 5 mm zone increase in the presence of cloxacillin, without a corresponding increase in its absence

| Disc             | Without |   | With cloxacillin |   |
|------------------|---------|---|------------------|---|
| Ceftazidime      | 7       |   | 16               |   |
| Ceftazidime + CA | 10      | 3 | 24               | 8 |
| Cefotaxime       | 11      |   | 17               |   |
| Cefotaxime + CA  | 12      | 1 | 24               | 7 |

Not standardized

# AmpC and ESBL Detection Discs



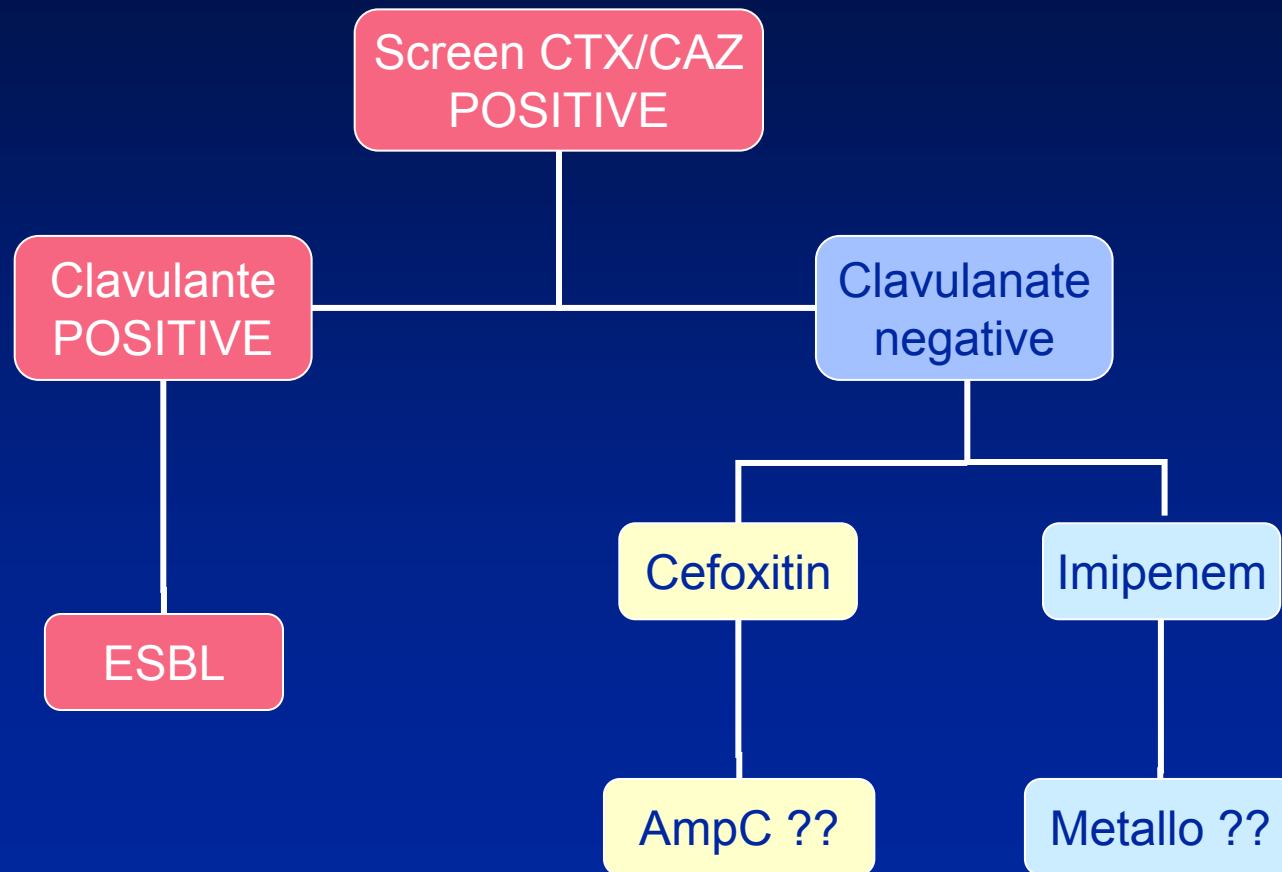
## MASTDISCS™ ID

Cefpodoxime 10 µg (A) with  
ESBL inhibitor (B), or AmpC  
inhibitor (C), or both (D)

- Neither ESBL or AmpC
- ESBL alone
- AmpC alone
- ESBL & AmpC combined

[www.mastgrp.com](http://www.mastgrp.com)

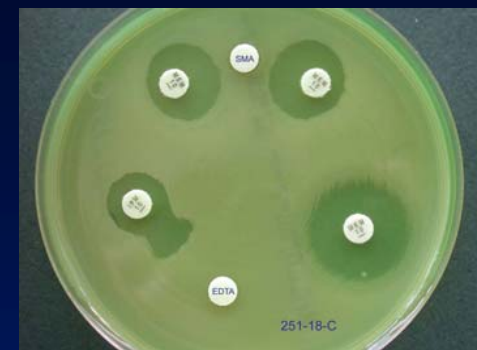
# ESBL, AmpC or metallo- $\beta$ -lactamase?



# Carbapenemase Detection?

- Media, media, media!!!!
- DDS test
  - MβLs inhibited with metal chelators  
(EDTA and thiol-based compounds)
- Modified Hodge Test
- Disk inhibitor test
- Inhibitor panels (false positive)
- Etest

# Phenotypic Tests



Which substrate?

Imipenem, meropenem, ceftazidime

What chemical?

EDTA

mercaptoacetic acid (SMA)

mercaptopropionic acid (MPA)

Sounds familiar!



Commercial tests:

ETest – insensitive with *Enterobacteriaceae*

Rosco tablets: *IMI+EDTA*

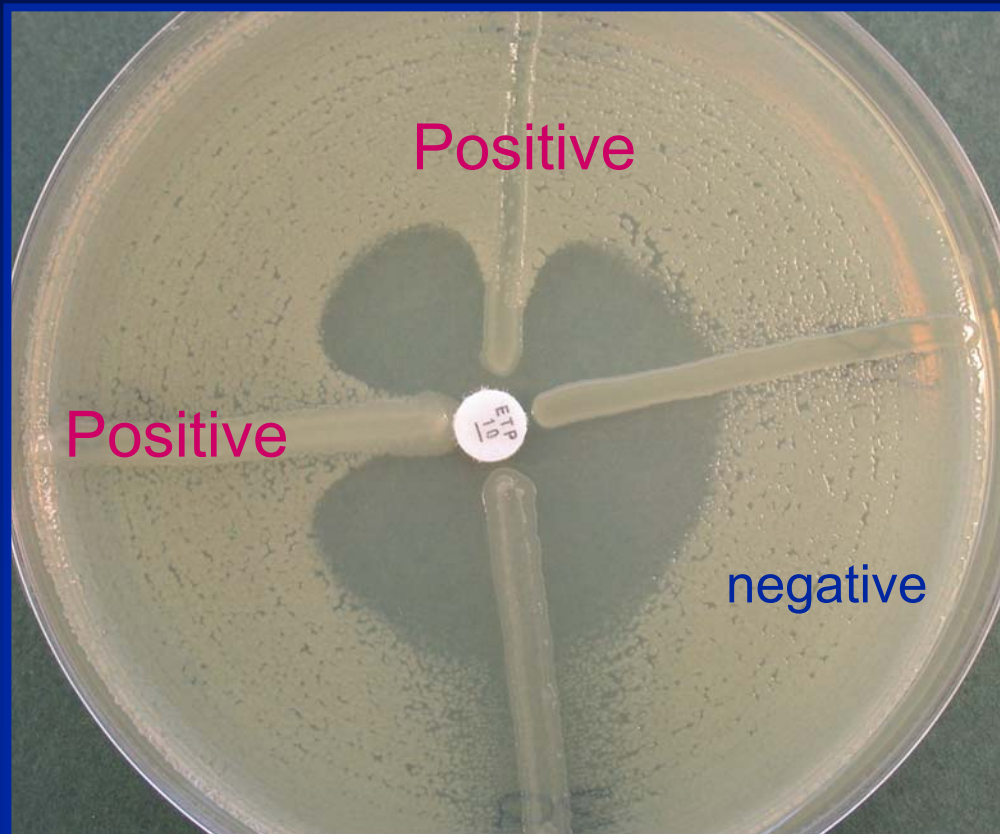
# KPC

“*Klebsiella pneumoniae* carbapenemase”

- Found on transferable plasmids
- Substrate hydrolysis includes aminothiazoleoxime cephalosporins such as cefotaxime
- Predominantly found in *K. pneumoniae*
  - *Enterobacter* spp, *Salmonella*
- Negative MBL DDST

Not detected in Australia (YET)

# Phenotypic confirmatory test for carbapenemases in Enterobacteriaceae



Distorted inhibition zones

## Modified Hodge Test

- Mueller-Hinton Agar
- *E. coli* ATCC 25922  
1/10 of 0.5 McFarland
- ERT10  $\mu\text{g}$
- Streak test 3 isolates from edge of disk to periphery

CLSI (2009) M100-S19

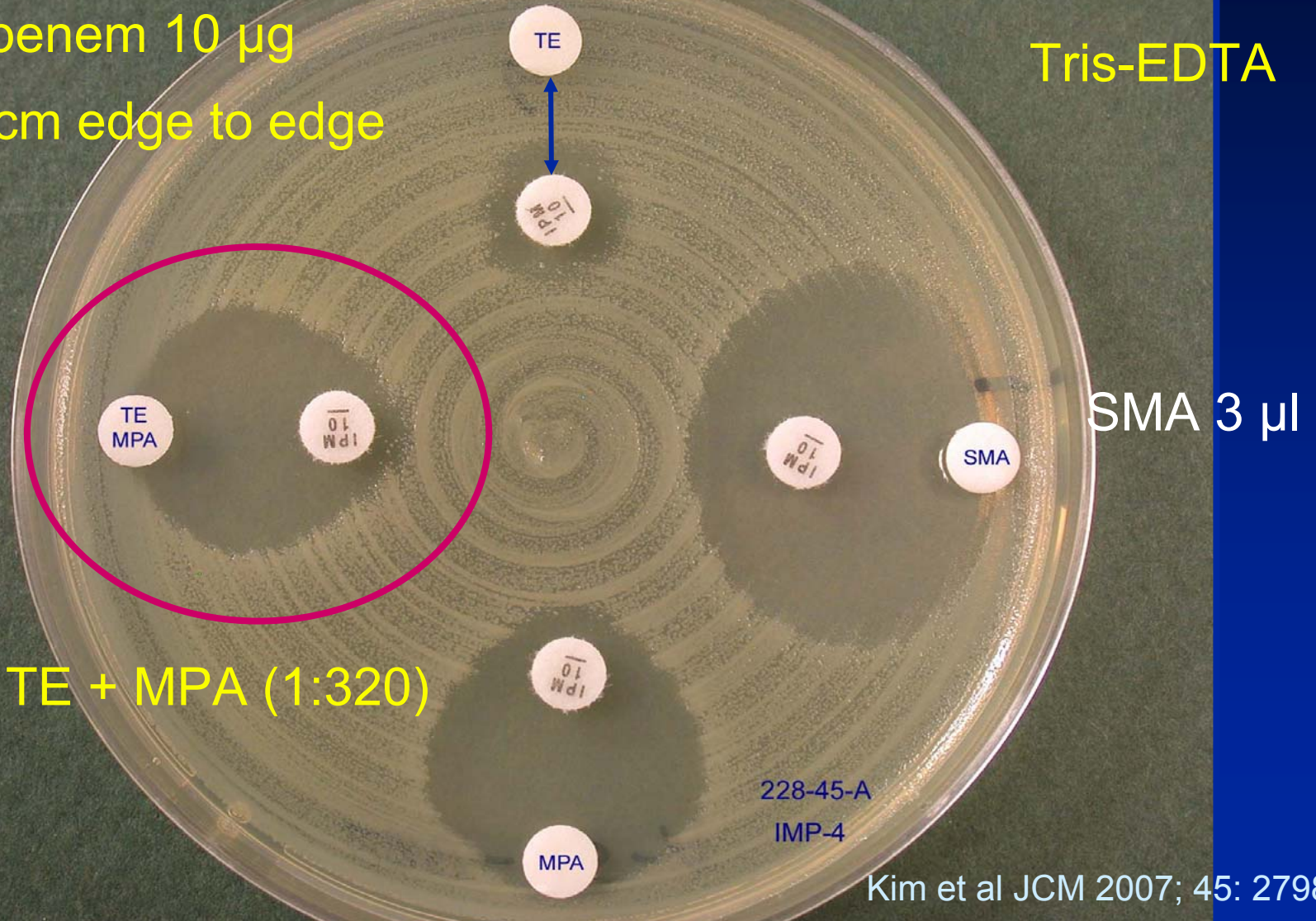
## “Convenient” DDST Test

- Imipenem based test with TE + MPA (1:320) most sensitive (100%) and specific (100%)
- TE alone did not detect 2 IMP-1 *A. baumannii* and 1 SPM-1 *P.aeruginosa*
- MPA alone failed to detect 1 VIM-2-like *P. aeruginosa*

# Convenient DDST Test

imipenem 10  $\mu$ g  
10 cm edge to edge

Tris-EDTA



Kim et al JCM 2007; 45: 2798

# CICA- $\beta$ -Test (MAST)

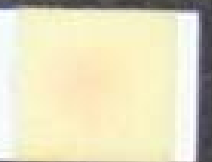
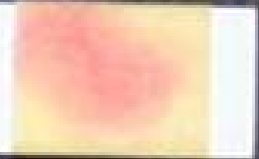
HMRZ-86

**No inhibitor**

**Mercaptoacetic acid to inhibit MBL**

**Clavulanate to inhibit ESBL**

**Boronic acid to inhibit AmpC**



## Detection Issues

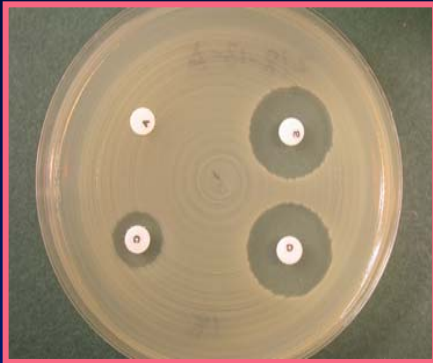
- Many *Enterobacteriaceae* with carbapenemase activity will have “sensitive” MIC or zone diameters
- Many organism have multiple enzymes, and are often multi-drug resistant
- Changes to standard guidelines
- Direct phenotypic detection difficult

## What should we look out for?

|             | <i>P. aeruginosa</i> | <i>A. baumannii</i> | Coliforms |
|-------------|----------------------|---------------------|-----------|
| Cefotaxime  | High R               | High R              | R         |
| Ceftazidime | High R               | High R              | R         |
| Ertapenem   | R                    | R                   | I/R       |
| Imipenem    | R                    | S/I/R               | S/I/R     |
| Meropenem   | R                    | S/I/R               | S/I/R     |

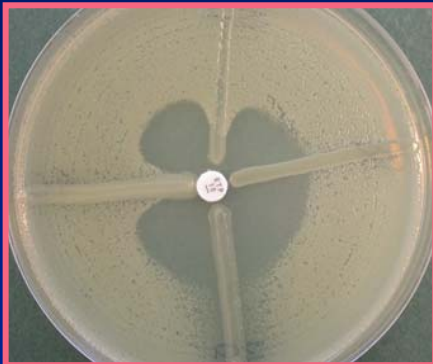
# Summary

- Labs should be able to recognise ESBL producers
  - Even among Enterobacters
  - Reference laboratory for difficult cases
- Labs should be able to recognise AmpC derepressed strains & those with plasmid AmpC
- Enterobacteriaceae with reduced carbapenem susceptibility need reference investigation
- New tests being developed; be aware of methodology changes



## ESBL

Including possible plasmid borne  
AmpC



## MHT

Positive: MBL (imp, vim, aim)  
Negative: KPC, OXA



## DDST

MBL (imp, vim, aim)  
KPC (MHT negative)  
OXA-48 (MHT negative)



# What to test?

## Reduced susceptibility to carbapenems

| Organism   | carbapenem                 |
|--|----------------------------|
| <i>E. coli</i> , <i>Klebsiella</i> spp.                              | $\geq 1$ mg/L <sup>a</sup> |
| <i>Enterobacter</i> spp., <i>C. freundii</i> or <i>S. marcescens</i> | $\geq 4$ mg/L              |
| <i>P. aeruginosa</i>   | $\geq 4$ mg/L              |
| <i>Acinetobacter</i> spp.  | $\geq 4$ mg/L              |

imipenem, meropenem

<sup>a</sup> etapenem

# Optimal conditions

| Group                     | Test | IMBL                           | Substrate   | Conditions         |
|---------------------------|------|--------------------------------|-------------|--------------------|
| <i>Enterobacteriaceae</i> | CD   | 10 $\mu$ l<br>EDTA<br>(100 mM) | imipenem    | Breakpoint<br>5 mm |
| <i>P. aeruginosa</i>      | DDST | 5 $\mu$ l<br>MPA<br>(1.4 mM)   | ceftazidime | Distance<br>2 cm   |
| <i>Acinetobacter</i> spp. | DDST | 5 $\mu$ l<br>MPA<br>(1.4 mM)   | imipenem    | Distance<br>2 cm   |

Picão et al (2008) JCM 46:2028-2037