WELCOME

to the

CDS WORKSHOP

Sydney 2010

http://web.med.unsw.edu.au/cdstest/

Frame Free CDS site

The CDS Antibiotic Susceptibility Test

Welcome to the CDS site

Full Window

Note: We are having problems with website registrations since the migrations to the new web server in July 2009. Online registrations submitted on or after the 21/06/2009 were not recorded. If you have registered since this date, are intending to register now or to change your registration details, please email *your* ***Name, Laboratory, Delivery and/or Mailing address and Telephone number*** to cds@sesiahs.health.nsw.gov.au

This site is maintained as an on-line resource for laboratories that perform antibiotic susceptibility testing. It was designed primarily for those who use the CDS method of testing but will be of interest to all who perform antibiotic susceptibility testing irrespective of the method usd.

The CDS test is a high potency disc diffusion method of antibiotic susceptibility testing where the results are calibrated to an internationally accepted quantitative technique. It was developed in the late 1960s and made available to laboratories in Australia in an effort to improve their performance in susceptibility testing. The CDS is supported by the CDS Reference Laboratory based at Randwick NSW. The laboratory constantly updates the CDS method to include testing of recently released antibiotics and is modified, where necessary, to enhance the demonstration of newly detected mechanisms of resistance.

Excel Spreadsheet for Registration

Contact	Titl	First	Las	Positi	SubSection	Comp	AddressPreamble	Address	AddressSubu	Adc	Addr	Phone	Fax	Email
Sydney Bell	Prof	Sydney	Bell	Director	Department of Microbiology	SEALS	Level 4 Campus Centre	Barker St	RANDWICK	NSW	2031	02 93829054	(02) 9382 9098	smbell@unsw.edu.au

Recent Additions to the CDS

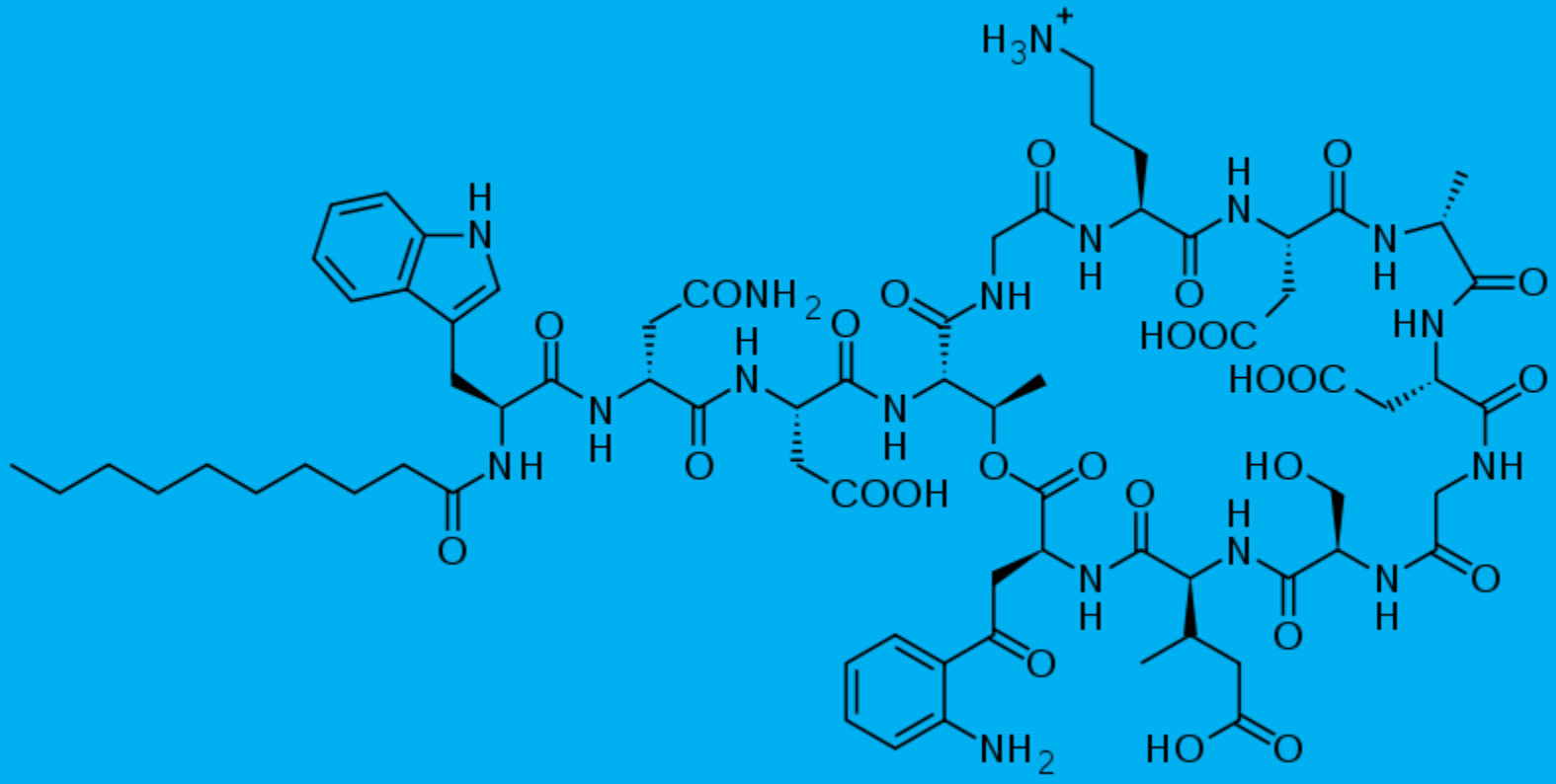
- Doripenem 10μg disc

A carbapenem claimed to be more active against Pseudomonas than Meropenem

- Daptomycin: see next slides – work in progress

Daptomycin

Lipopeptide

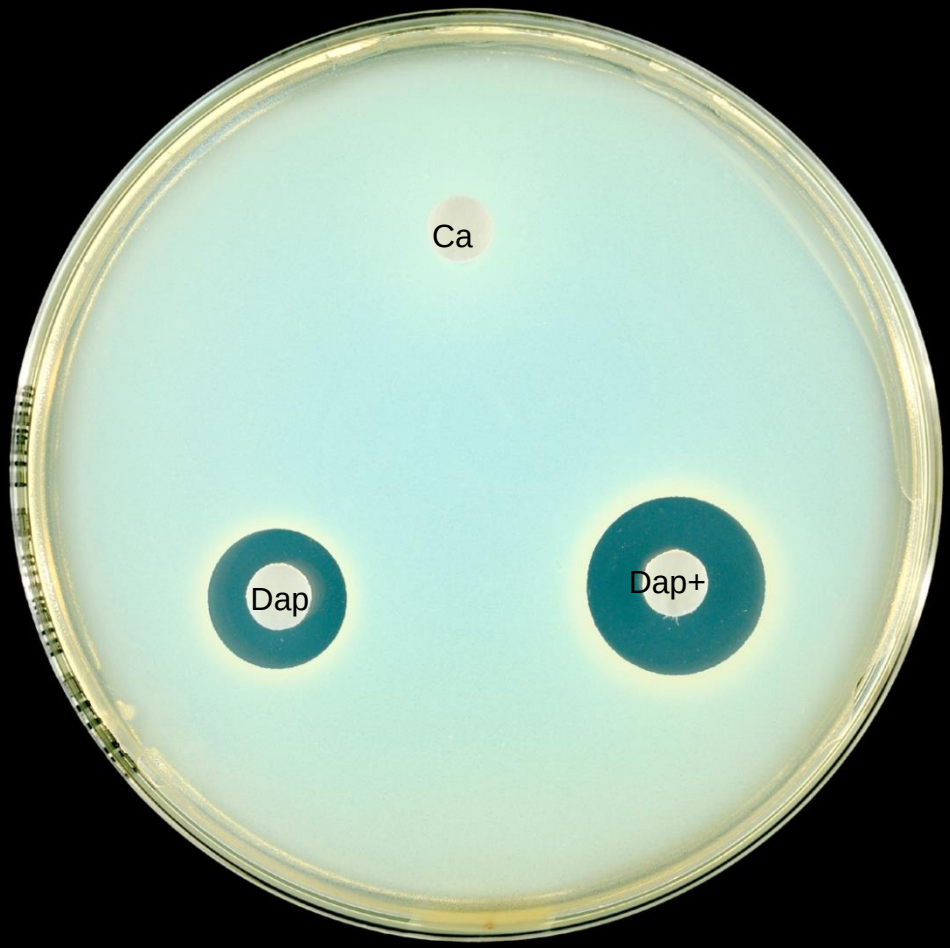


Daptomycin

Active against Gram positive organism including VRE

Susceptible strains have an MIC ≤ 4 mg/L

Testing *in vitro* requires 50 mg/L Ca^{++} in medium

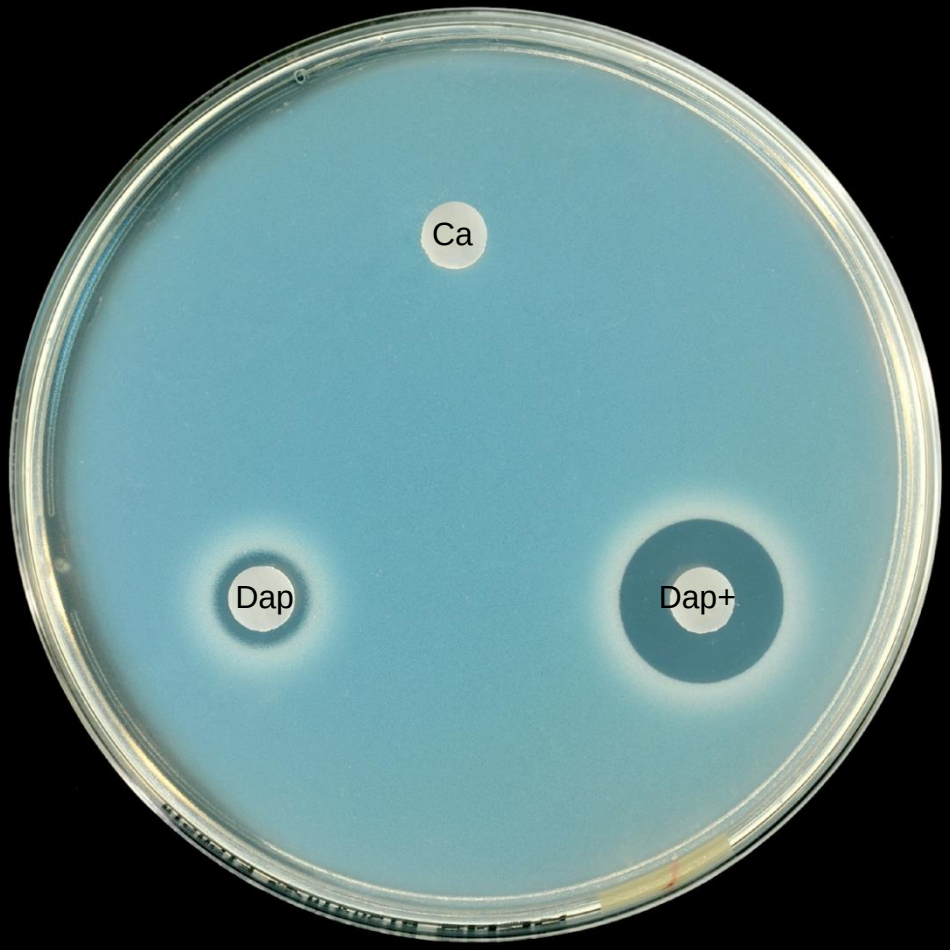


S. aureus ACM 5190 reference strain

Dap + = daptomycin 30 µg + $CaCl_2$ 250 µg disc (an. radius 5mm)

Ca: $CaCl_2$ 250 µg disc

Dap: daptomycin 30 µg disc

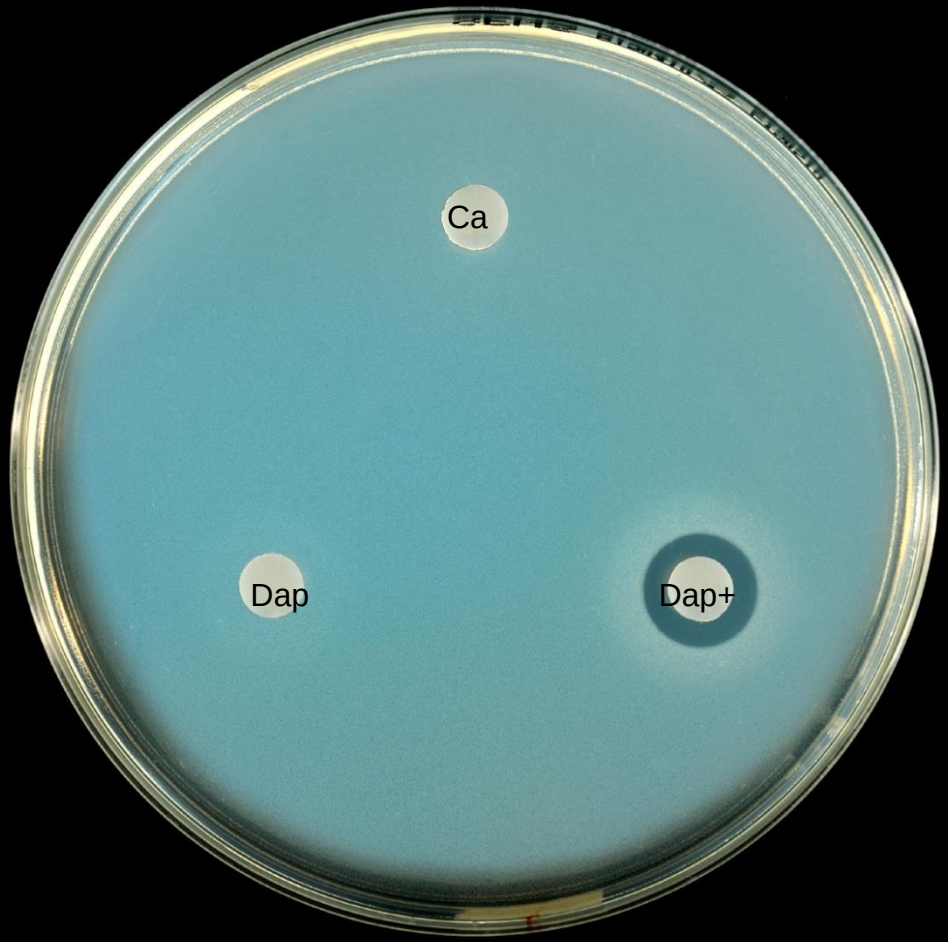


E. faecalis ACM 5184 on Sensitest agar in air

Dap + = daptomycin 30 µg + $CaCl_2$ 250 µg disc (an. Radius 5mm)

Ca: $CaCl_2$ 250 µg

Dap: daptomycin 30 µg



A strain of VRE faecalis of Van A phenotype resistant to Daptomycin (MIC 16 mg/L)

Dap + = daptomycin 30 µg + CaCl2 250 µg (an. radius 2 mm)

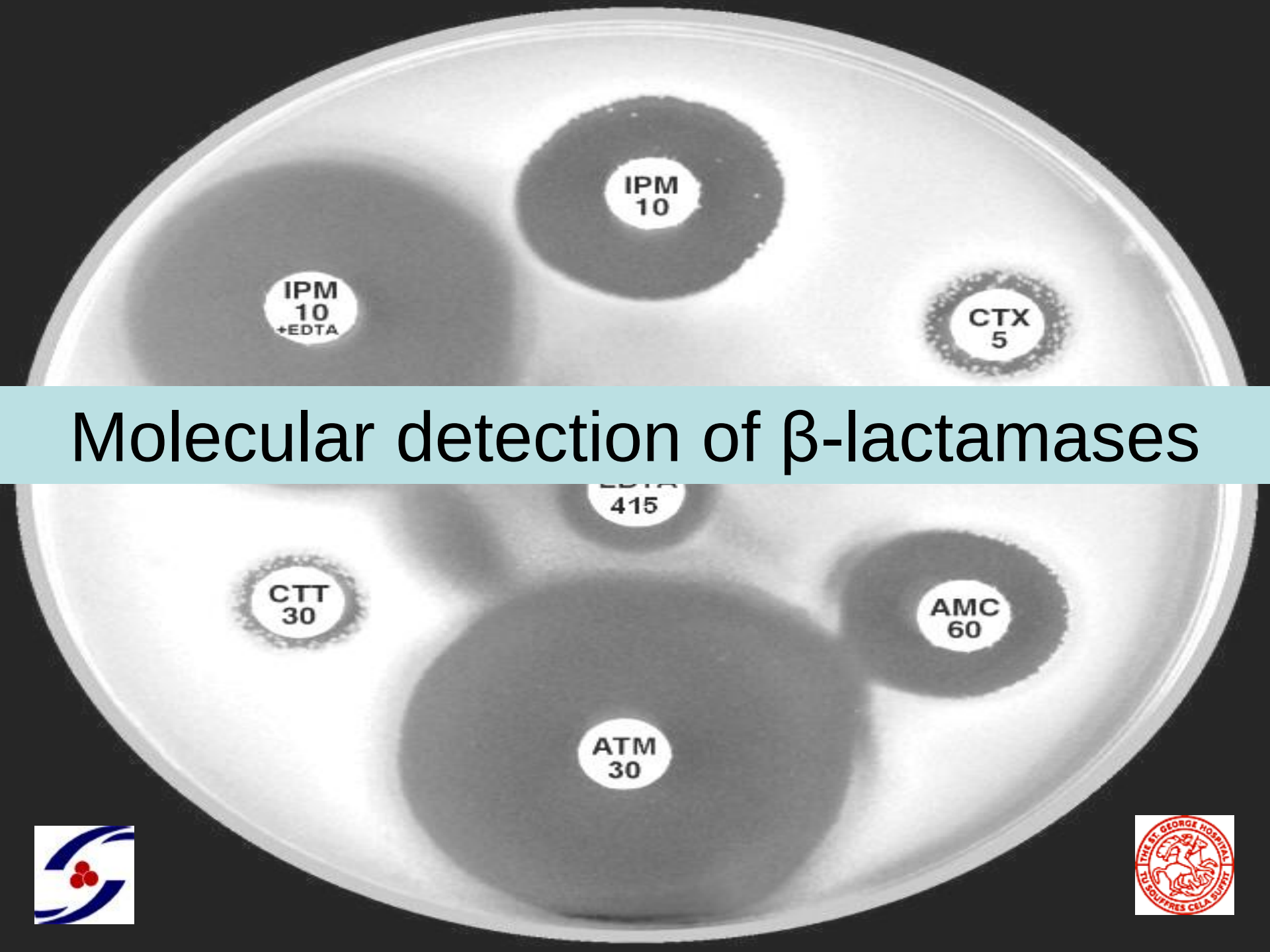
PCR Detection Of Putative Markers of β–Lactamases

Presentation by Dr. Chris McIver

An update of the CDS test

CDS Workshop

ASM 2010 Sydney



Molecular detection of β-lactamases

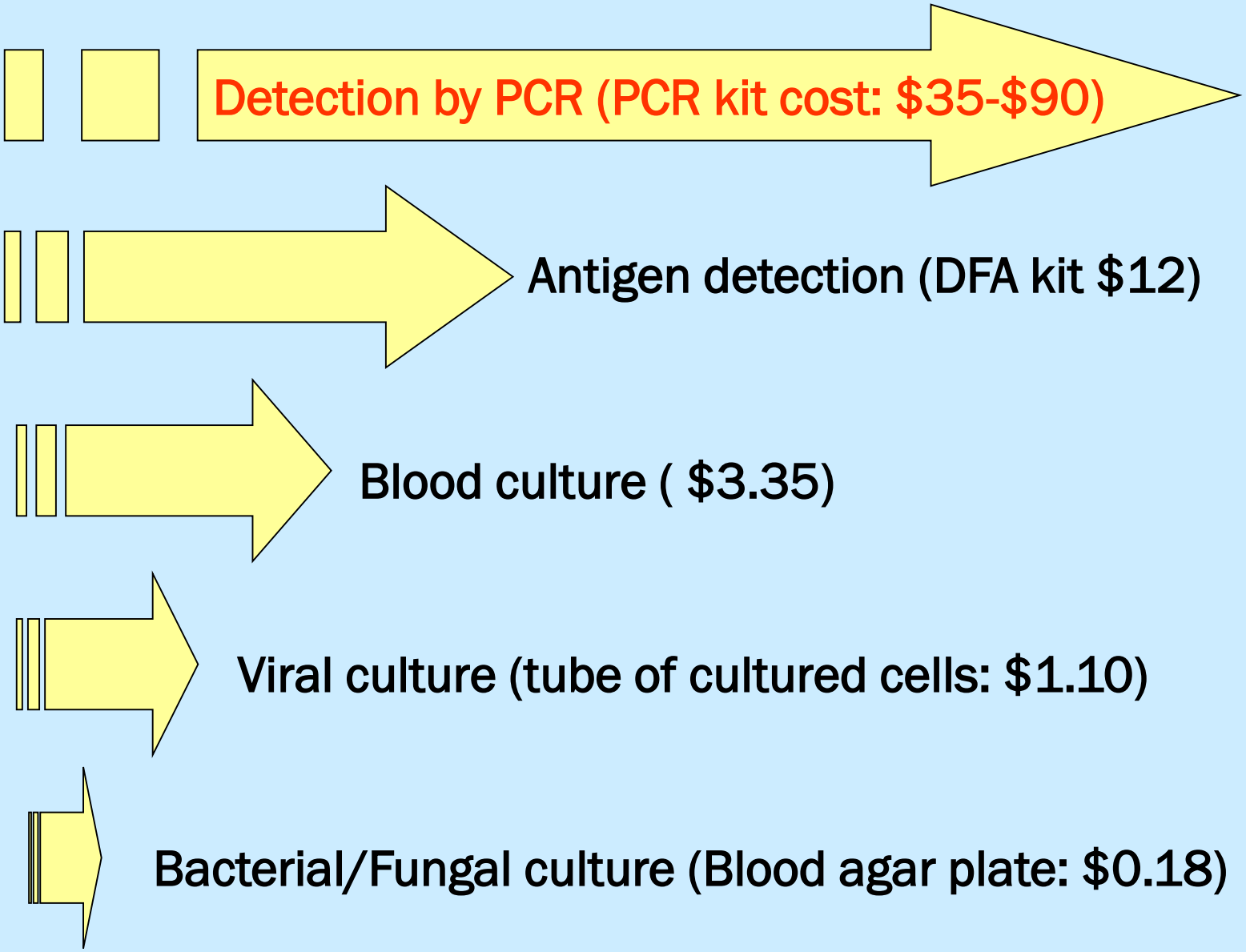
Outline

Imperatives for molecular detection

Applications in SEALS

Multiplex assay for common β-lactamases

- CTX-M
- Metallo-β-latamase (MBL) genes
- *Klebsiella pneumoniae* carbapenenase (*bla*KPC)
- OXA-23 Carbapenem-hydrolyzing β-lactamase



Hayden RT. 2004. Molecular Microbiology. Diagnostic Principles and Practice. ASM

Rapid detection = early intervention = reduce generation rate

$$R_0 = \beta c D$$

β = infectivity (probability of infection)

c = interaction rate between susceptible and infected

D = duration of infection

Brunham & Plummer. 1990. Med Clin Nth Am 74:1339-1352

$$R_0 = \beta c D$$

β = infectivity (probability of infection)

Patient-to-patient transmission
Contaminated hands, clothing, equipment of healthcare workers

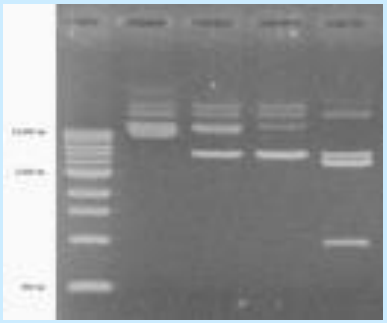
C = interaction rate between susceptible and infected

Cohort nursing
Contact isolation
Barrier nursing

D = duration of infection

Decolonisation treatment

Applications



- Integrons
- Quinolone resistant determining regions
- Vancomycin-resistant enterococci
- Methicillin-resistant *Staphylococcus aureus*
- β-lactamases

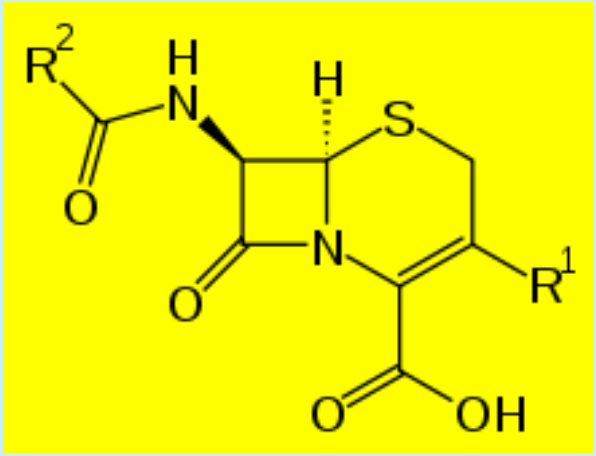
Indications for molecular detection

- **Clarification of phenotypic morphology**
- **Direct testing on clinical specimens**
- **Surveillance**






Ambler classes of β-lactamases



Oxyimino-cephalosporin

TEM
SHV
CTX-M

B-lactamases

Serine active site

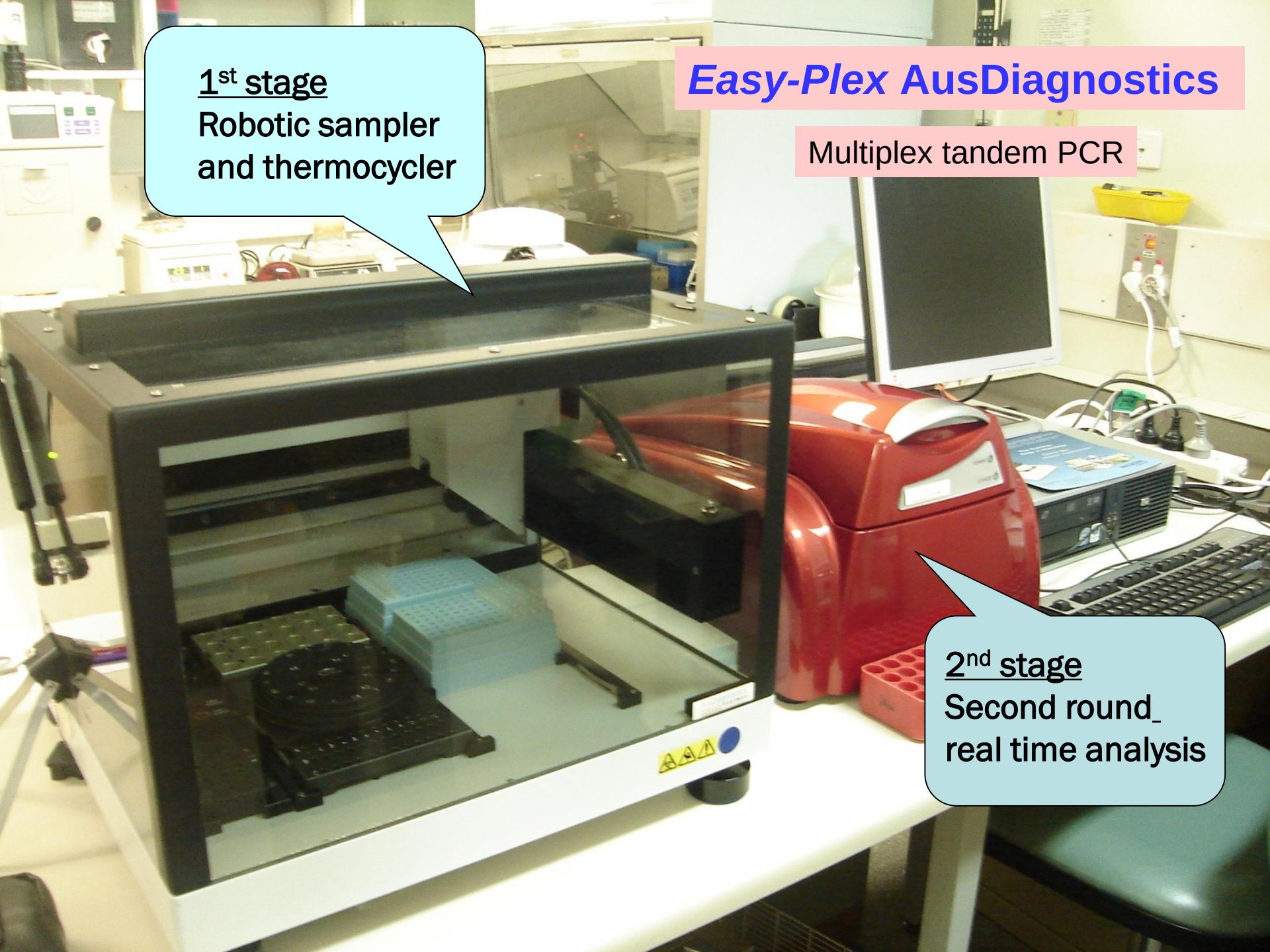
Class B (metallo)

Class A

Class C
AmpC

Class D
OXA

Hall and Barlow. 2005. J. Antimicrob. Chemoth.


1st stage
Robotic sampler
and thermocycler
Easy-Plex AusDiagnostics
Multiplex tandem PCR
2nd stage
Second round_
real time analysis

Easy-Plex AusDiagnostics

nuc *S. aureus* thermostable nuclease
mecA methicillin-resistance
SCC staphylococcal cassette chromosome

vanA vancomycin resistance gene A
vanB vancomycin resistance gene B

CTX-M group 1 Plasmid beta-lactamase bla-CTX-M-1
CTX-M group 9 Plasmid beta-lactamase bla-CTX-M-9

pan-vim Metallo-β-lactamase vim1, 2 or 3
pan-IMP Metallo-β-lactamase 1 and 4

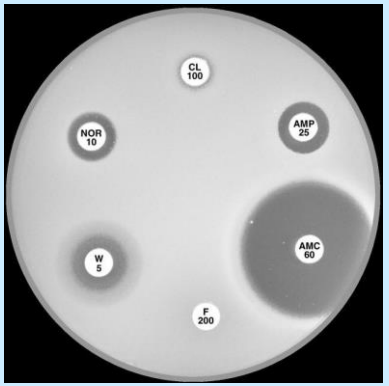
KPC *Klebsiella pneumoniae* carbapenemase

Oxa-23 Carbapenem-hydrolysing β-lactamase

OXA-23 Carbapenem-hydrolysing β-lactamase

2010 strains	OXA-23
Acinetobacter	3

Strains	OXA-23
Acinetobacter	4



Carbapenems have become the drugs of choice against *Acinetobacter* infections

Emergence of carbapenem-hydrolysing β-lactamases of molecular Classes B and D

Class B carbapenemases found so far in Acinetobacters include:
various IMP and VIM types

Class D enzymes including members of the OXA-23- and OXA-24

Klebsiella pneumoniae carbapenenase (bla_{KPC})

Strains	KPC
Klebsiella pneumoniae	**5**

Plasmid-mediated

Confers resistance to all β-lactam agents including: carbapenems

Can co-exist with other Gram–negative resistance mechanisms including:
ESBL, fluoroquinolone, and aminoglycoside resistances

CTX-M family comprises 40 enzymes:

Class A

CTX-M-1 group (six plasmid-mediated enzymes)

CTX-M-1; CTX-M-3; CTX-M-10; CTX-M-12;
CTX-M-15; FEC-1
Unpublished enzymes:
CTX-M-22; CTX-M-23; CTX-M-28

CTX-M-9 group (nine plasmid-mediated enzymes)

CTX-M-9; CTX-M-13; CTX-M-14; CTX-M-16; CTX-M-17;
CTX-M-19; CTX-M-21; CTX-M-27; Toho-2
Unpublished enzymes:
CTX-M-24; CTX-M corresponding to Accession No. JP0074

Bonnet, R. 2004. *Antimicrobial Agents and Chemotherapy*. **48:**1-14.
Zong et al. 2008 *Antimicrobial Agents and Chemotherapy*. 52:4198 - 4202

Clinical *ESBL* isolates (2010)

n = 39/42

Class A

Strain	n	CTX-M-1	CTX-M-9	Other
Coliform	2	0	0	0
Citrobacter sp	1	0	0	0
Escherichia coli	30	11	17	2
Klebsiella sp	7	5	1	1
Enterobacter cloacae	1	0	1	pan-IMP
Enterobacter sp	1	0	0	0

bla $_{\text{CTX-M}}$ in archival and recent isolates of *Escherichia coli* and *Klebsiella*

Region	CTX-M-1	CTX-M-9	n
East Sydney SEALS	25	26	51
West Sydney ICPMR*	38	23	61

$p = 0.1790$

*Zong et al. 2008 AAC. 52:4198 - 4202

Clinical MBL isolates (2010)

n = 7

Strain	n	pan-IMP	pan-VIM	Other
Citrobacter sp	1	1	0	0
Enterobacter cloacae	1	1	0	CTX-M-9
Enterobacter cloacae	1	1	0	0
Klebsiella pneumoniae	1	1	0	0
Klebsiella oxytoca	1	1	0	0
Pseudomonas sp	2	1	1	0

Detection of MBL genes in archival and recent isolates

n = 17

Strain	n	pan VIM	pan IMP	Other
Escherichia coli	1	0	1	0
Klebsiella sp.	7	0	7	0
Enterobacter aerogenes	1	0	1	CTX-M-9
Enterobacter cloacae	1	0	1	CTX-M-9
Enterobacter sp.	3	0	3	0
Citrobacter sp.	1	0	1	0
Pseudomonas sp.	3	2	1	0

Concluding notes

Phenotypic tests: prediction of *in vivo* response

Multiplex platform allows convenient broad spectrum screen

Prevalence of important determinants

Applicable to cultures and infections of sterile body sites

Early detection of emerging resistance determinants

$$R_0 = \beta c D$$

Acknowledgement

Prof. Sydney Bell

Dr Jeanette Pham

Ian Carter

Microbiology Department (SEALS), St George Hospital

Staphylococci v/s cefoxitin 10/ oxacillin 1

Standard 6 mm cut off

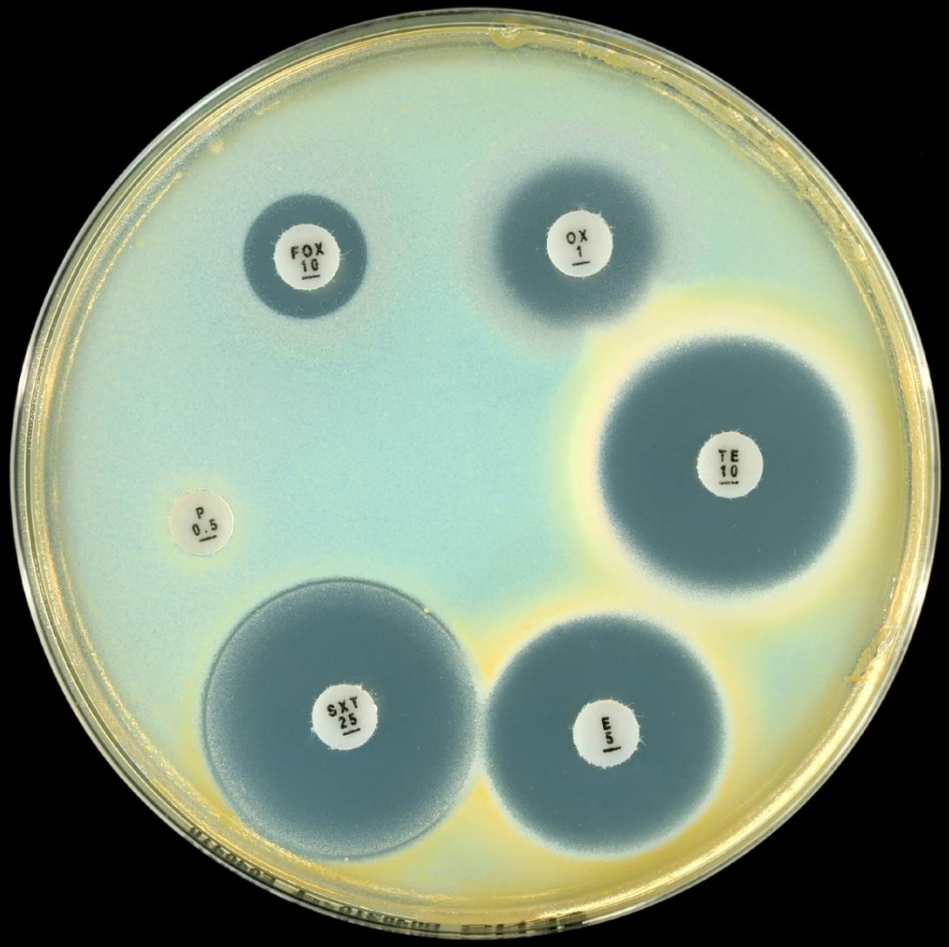
Cefoxitin 10 (Fox 10) for *S. aureus*

* No problem with BORSA (MSSA with high penicillinase activity)

Oxacillin 1 (Ox 1) for CNS

Excellent correlation with *mecA* gene PCR

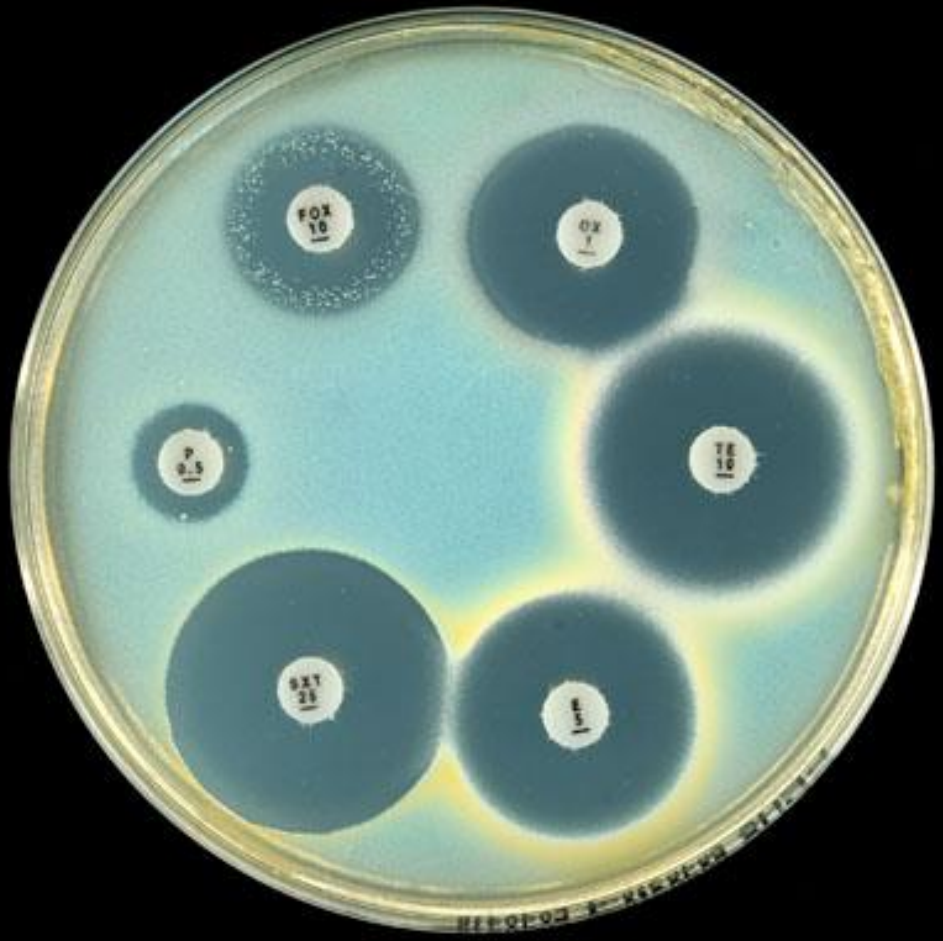
Report S or R to methicillin



Non multi-resistant MRSA = original CA-MRSA

R/ penicillin (P 0.5) and cefoxitin (FOX 10)

S/ tetracycline (TE 10), erythromycin (E 5), co-trimoxazole (SXT 25)



Oxacillin-susceptible *mec-A* positive *Staphylococcus aureus*

Non multi-resistant MRSA with a heterogeneous resistance to methicillin

=> numerous resistant colonies in FOX 10 zone, large OX 1 zone

=> cefoxitin is a better inducer of PBP 2a than oxacillin

The ß-lactamases of Gram-negative bacilli

The ß-lactamases of Gram-negative bacilli

Acinetobacter v/s ampicillin/cephalexin

Acinetobacter lwoffi (-like)

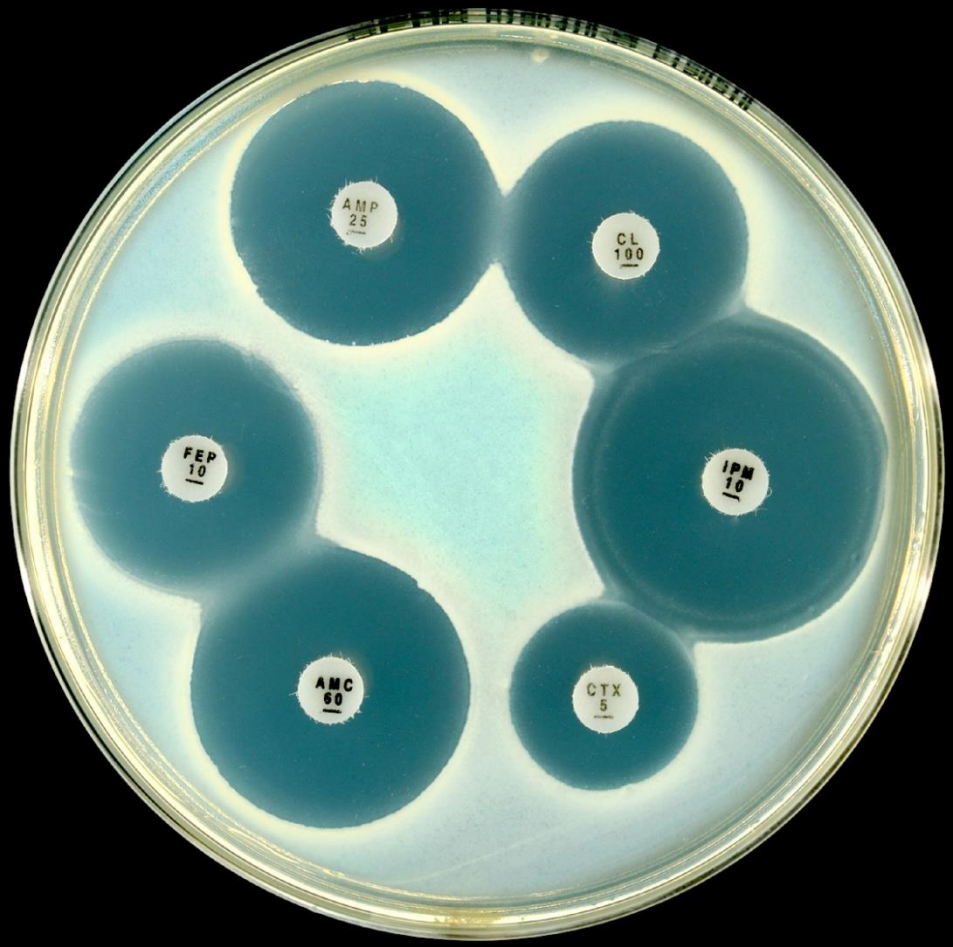
β-lactamase negative → S/ ampicillin, cephalexin

Acinetobacter baumanii (-like)

Non-inducible chromosomal cephalosporinase of AmpC type

→ R/ ampicillin, cephalexin

Resistance to cephalexin = marker of AmpC



A typical *Acinetobacter lwoffi*:

S/ ampicillin (AMP 5) and a cephalexin (CL 100)
* A slightly reduced CTX 5 zone

A typical *A. baumanii (or A. baumanii-like)*:

R/ ampicillin (AMP 5) , cephalexin (CL 100), cefotaxime (CTX 5), cefepime (FEP 10)

S/ imipenem (IPM 10)

An unusually low cephalosporinase activity *A. baumanii (or A. bauminii-like)*
R/ CL 100 (cephalexin), AMP 25 zone > 6 mm with colonies at edge

Report: R/ ampicillin, cephalexin, cefotaxime
S/ cefepime, Augmentin, imipenem

Multiple resistant *Acinetobacter baumanii*

- Resistant to various classes of antibiotic
- Resistant to all β-lactams including carbapenems

(Ambler class D = oxacillinases… Oxa-58)

- Susceptible to polymyxin-B

The ß-lactamases of Gram-negative bacilli

An update

on the detection of plasmid mediated

β-lactamases in clinical isolates in Australia

Common transferable (plasmid mediated) β-lactamases in coliforms

- TEM-1, SHV-1, ESBLs (Bush group 2, Ambler class A)

  Inhibited by CA

  S/ AMC 60

- AmpC: (Bush group 1, Ambler class C)

  Not inhibited by CA, inhibited by boronic acid

  R/ AMC 60 S/ FEP 10

- MBL: (Bush group 3, Ambler class B)

  Not inhibited by CA, inhibited by EDTA

  R/ AMC 60 R/ FEP 10

ESBLs *sensu-stricto*

(Ambler class A, Bush group 2)

Inhibited by CA

R/ Cephalosporins (including cefepime) and aztreonam

S/ Augmentin (AMC 60)

S/ Cephamycin (cefoxitin, cefotetan)

CDS routine testing → Synergy with AMC 60

(no need for confirmation)

S/ Imipenem (T)

Disc positions recommended for routine testing

Klebsiella pneumoniae producing an ESBL: synergy between Augmentin (ACM 60) and cefepime (FEP 10), no obvious synergy with cefotaxime (CTX 5) due to high activity of ESBL.

Detection of PM-AmpC in *E. coli*

R/ AMC 60 (not inhibited by CA)

R/ CL 100

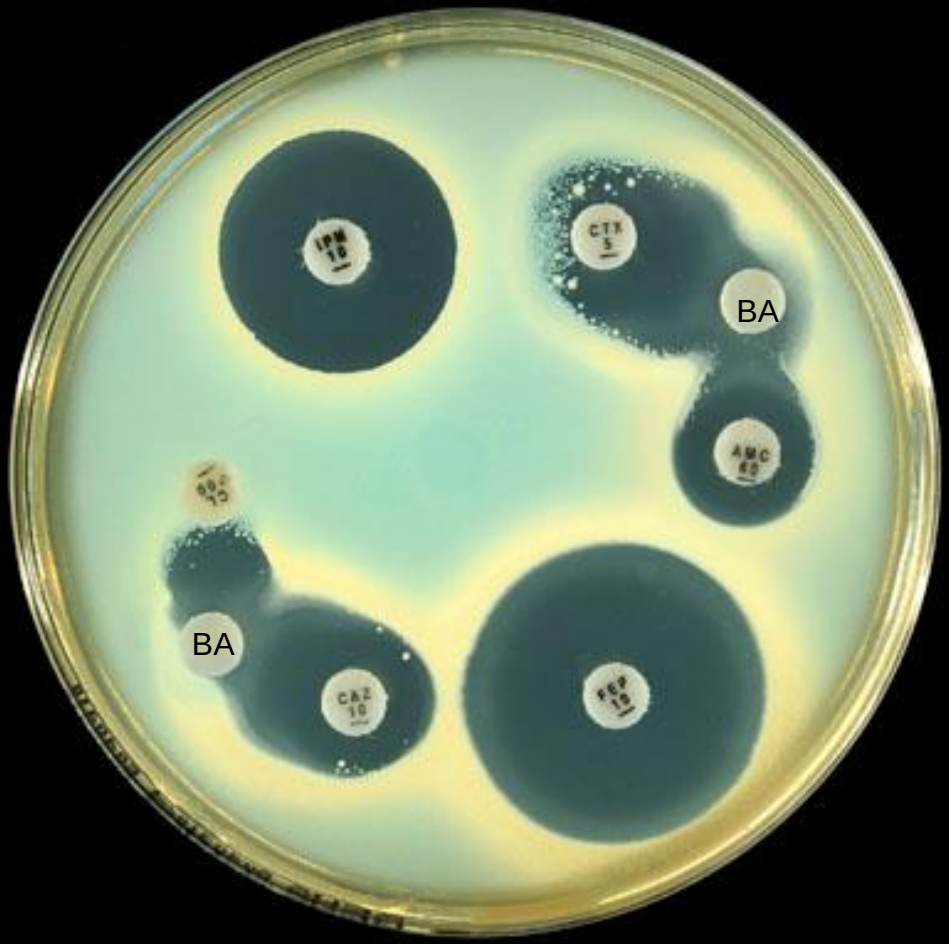
R/ CTX 5 (high level resistance)

R/ cefamycin (CMY-1…)

S/ FEP 10

Confirmation (optional): inhibition by boronic acid (BA)
(1-Benzothiophene-2-boronic acid)

Routine CDS test showing an *E. coli* with plasmid mediated AmpC (PM AmpC)
R/ Augmentin (AMC 60), cephalexin (CL 100), cefotaxime (CTX 5);
Key markers: S/ cefepime (FEP 10) and imipenem (IPM 10).



The same *E. coli* with PM AmpC S/ IPM, FEP

Synergy between boronic acid discs (BA) and adjacent discs:

Cefotaxime (CTX 5), Augmentin (AMC 60), cephalexin (CL 100), ceftazidime (CAZ 10).

BA= 250 µg boronic acid disc

Acquired Metallo-Beta-Lactamases (MBLs)

Ambler class B or Bush group 3

Inhibited by EDTA (Zinc molecule)

IMP-4 (most common), NDM-1

VIM, SPM, GIM, SIM (*P. aeruginosa*)

Hydrolyses all beta-lactam (except aztreonam)

Enterobacteriaceae

May have a zone > 6mm with IPM 10

Pseudomonas aeruginosa (pigmented)

Highly resistant to all β-lactams => no zone

Susceptible to aztreonam (S/ ATM)

E. coli: R/AMP 25, AMC 60, CTX 5, CL100 and FEP 10, colonies at the edge of imipenem zone (> 6 mm).

No synergy between FEP/AMC → not ESBL => ? MBL

Resistant colonies at the edge of IPM 10 zone => ? MBL

Confirmatiom:

- Synergy between EDTA (blank disc= EDTA 415 µg) and imipenem (IPM 10), cefotaxime (CTX 5) ertapenem (ERP 10), cefepime (FEP 10)
- S/ ATM (aztreonam)

=> Metallo-β-lactamase

E. coli: R/AMP 25, AMC 60, CTX 5, CL100 and FEP 10, colonies at the edge of imipenem zone (> 6 mm).

No synergy between FEP/AMC → not ESBL => ? MBL

Resistant colonies at the edge of IPM 10 zone => ? MBL

Confirmatiom:

- Synergy between EDTA (blank discs) and IPM 10, ETP 10 only
- R/ ATM and synergy with AMC 60

=> MBL and ESBL

KPC in *Klebsiella pneumoniae*

Plasmid mediated *K. pneumoniae* carbapenemase (KPC)

Ambler class A or Bush group 2f

Reported in Europe, US (Brooklyn 24%)

Inhibited by clavulanic acid => ESBL affecting carbapenems

KPC-1 , KPC-2,…KPC-4

High level resistance to FEP, CTX, CRO, CAZ, ATM, ….

Imipenem MIC ≥ 4 mg/L (zone > 6 mm with resistant colonies)

Ertapenem MIC > 8 mg/L (resistant)

Inoculum dependent => broth MIC unreliable

CDS: R/IPM or colonies at edge of IPM zone

Confirmation: No synergy with EDTA, R/ ertapenem

Mild synergy with AMC 60

Send to us for PCR confirmation

K. pneumoniae: R/ Augmentin (AMC 60), cephalexin (CL100), cefotaxime (CTX 5), cefepime (FEP 10), imipenem (IPM 10) zone (> 6 mm with numerous resistant colonies).

No synergy with EDTA ???

The same *K. pneumoniae:*

- No synergy with EDTA
- Synergy between AMC 60 and IPM 10 => inhibited by clavulanate

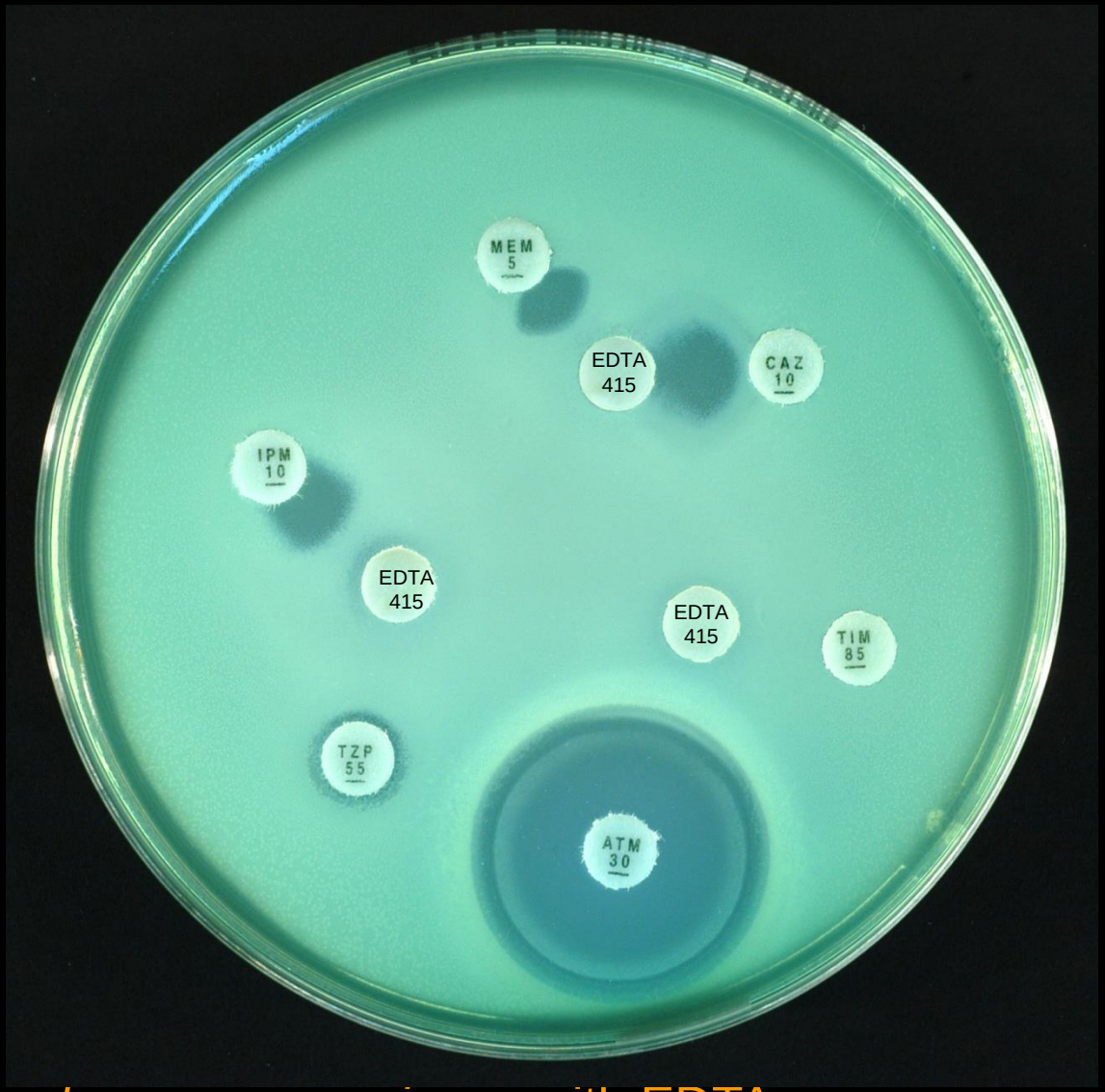
= > carbapenemase of Ambler class A or Bush group 2

KPC-2 producing *K. pneumoniae* from Greece

Pseudomonas aeruginosa (pigmented on Sensitest agar)

No zone around imipenem (IPM 10) ceftazidime (CAZ 10), tazocin (TZP 55), cefepime (FEP 10) and Timentin (TIM 85) S/ aztreonam (ATM 30)

=> Candidate for MBL detection



The same *Pseudomonas aeruginosa* with EDTA

Detection of MBL: Synergy between an EDTA disc placed next to imipenem (IPM 10)/ meropenem (MEM 5)/ ceftazidime (CAZ 10) discs.

S/ aztreonam (ATM 30)