Welcome

to

the CDS Workshop

Jeanette Pham

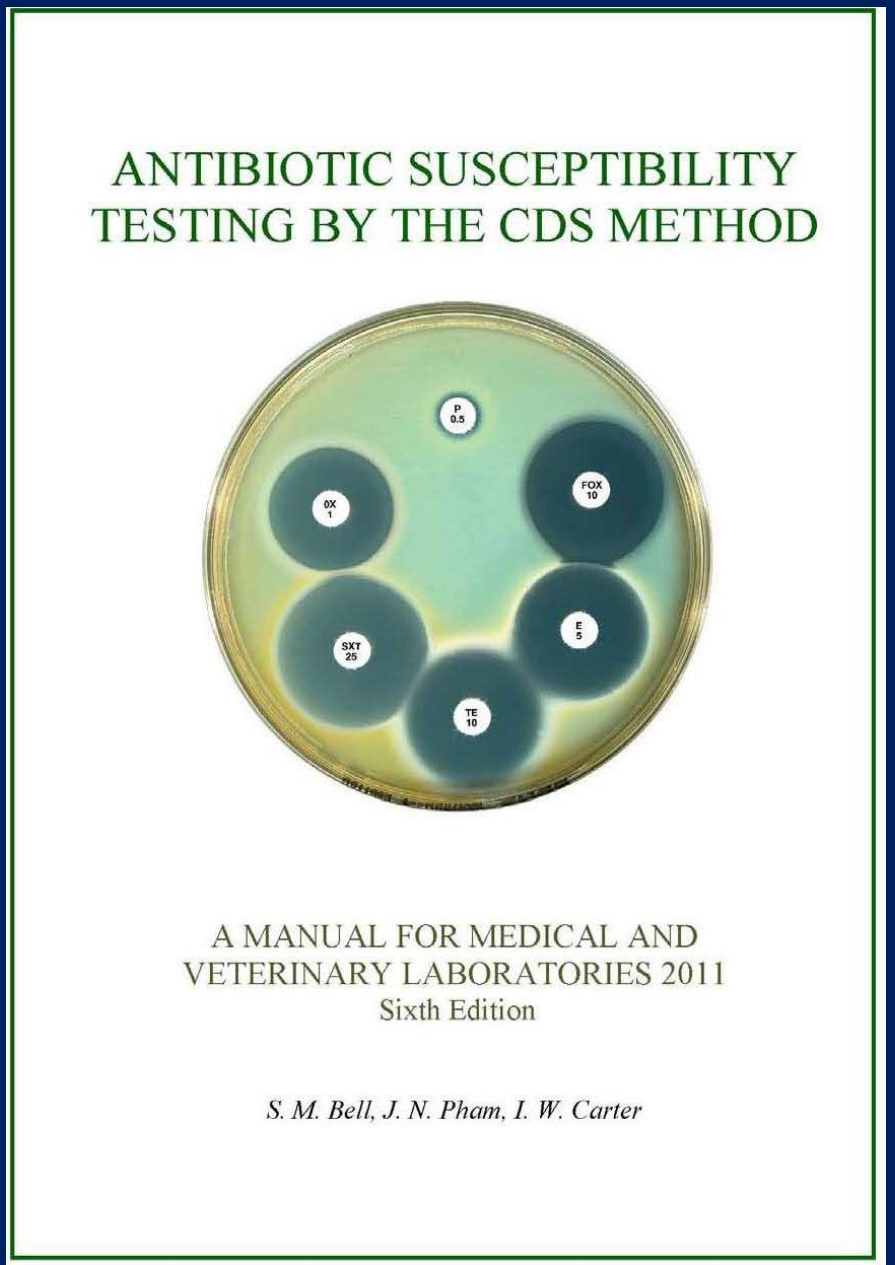
Syd Bell

Hobart 2011

VALE - Maria Yates

1954 - 2011

The Sixth Edition is Coming !



Help Needed !

If you have any suggestions on modifications

or

additions to the Manual

Please email us at:

cds@sesiahs.health.nsw.gov.au

smbell@iinet.net.au

Changed Cefotaxime/Ceftriaxone Testing for

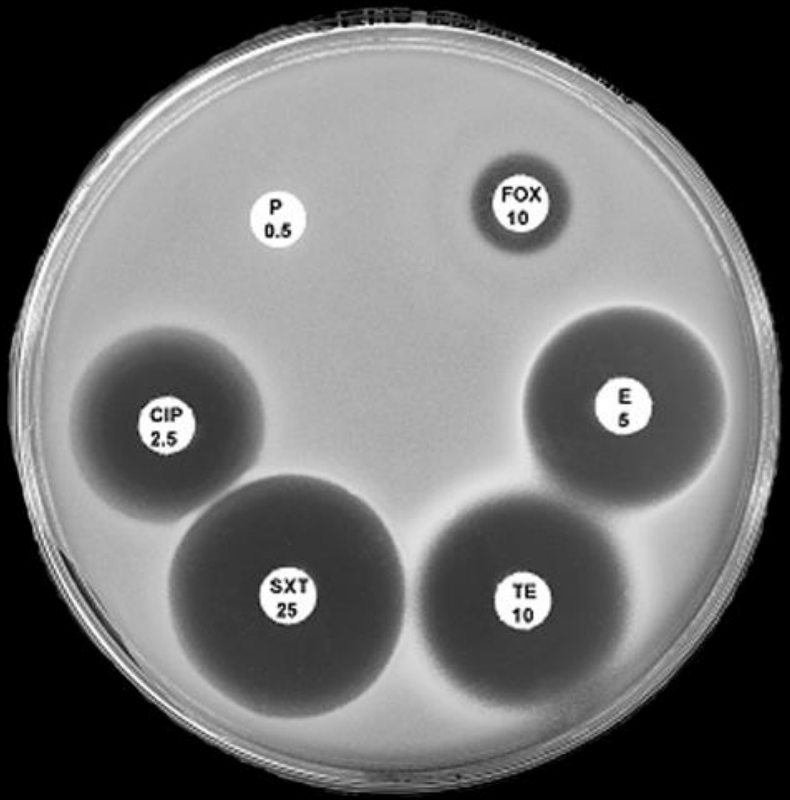
- Pneumococci
  (sites other than CSF)
- Streptococcus “milleri” group
  (anginosus, constellatus & intermedius)

Antibiotic/disc	Cut-off (mm)	MIC(mg/L)
Cefotaxime 0.5μg	4mm	0.5
Ceftriaxone 0.5μg	4mm	0.5

Susceptibility of “milleri” Group

Streptococcus pyogenes

Streptococcus “milleri”

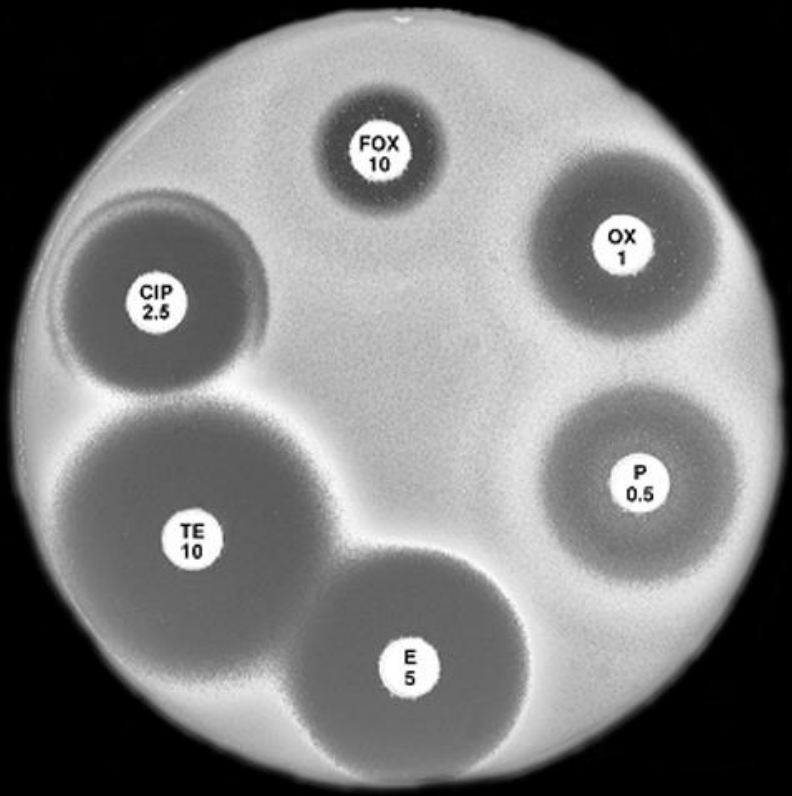


Non multi-resistant MRSA = original CA-MRSA

Resistant to penicillin (P 0.5) and cefoxitin (FOX 10)

S/ erythromycin (E 5), tetracycline (TE 10), co-trimoxazole (SXT 25) and ciprofloxacin (CIP 2.5) and gentamicin (not shown).

Recent isolates: R/ Ery, Cip.



β –Lactamase negative NMR- MRSA

Clear large zone around OX 1, light growth around P 0.5 and a small zone around FOX 10 => excellent inducer of PBP 2a.

Report as resistant to methicillin and all other β-lactams.

Staphylococus aureus v/s cefoxitin 10

Standard 6 mm cut off

***mecA* gene negative:** **zone around 7 - 9 mm**

***mecA* gene positive:** **zone 0 - 5 mm**

*** Rare strains have problem with expressing the resistance => zone around 6 mm**

*** Repeat with higher inoculum**

or

*** Send for *mecA* gene PCR**

Enterococci

Tested on blood Sensitest agar in 5% CO2
(high humidity in CO2 incubator enhances the growth)

Ampicillin 5 µg (AMP 5, 4 mm)
Gentamicin 200 µg (CN 200, high level resistance)
Linezolid 10 µg (LZD 10)
Nitrofurantoin 200 µg (F 200, urine, 4 mm)
Quinupristin/Dalfopristin 15 µg (QD 15)
(*E. faecalis*: naturally R/ QD)
Streptomycin 300 µg (S 300, high level resistance, 4 mm)
Teicoplanin 15 µg (TEC 15*)
Tigecycline 15 µg (TGC 15)
Vancomycin 5 µg (VA 5*)

*** Hazy edge with growth towards the discs**

Plate incubated in CO2:

Vancomycin resistant *Enterococcus faecium* of VanB phenotype

At 24 h, the reduced zone and the light growth towards the vancomycin disc (VA 5) is frequently observed with this phenotype.

R/ VAN S/ TEC => vanB phenotype

Plate incubated in Air:

Same VR *Enterococcus faecium* of VanB phenotype

At 24 h, the light growth towards the vancomycin disc (VA 5) is not obvious on the Sensitest agar plate incubated in air.

The ß-lactamases of Gram-negative bacilli

An update

on the detection of common plasmid mediated β-lactamases in clinical isolates

Common transferable (plasmid mediated) β-lactamases in coliforms

- **TEM-1, SHV-1, ESBLs (Bush group 2, Ambler class A)**
  Inhibited by clavulanic acid (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
  May have large zones
  R/ AMC 60 R/ FEP 10

- **KPC: "Super ESBL" (Bush group 2, Ambler class A)**
  Partially inhibited by CA
  R/ AMC 60 R/ ALL

***E. coli* ACM 5186 (used in QC) producing TEM-1**

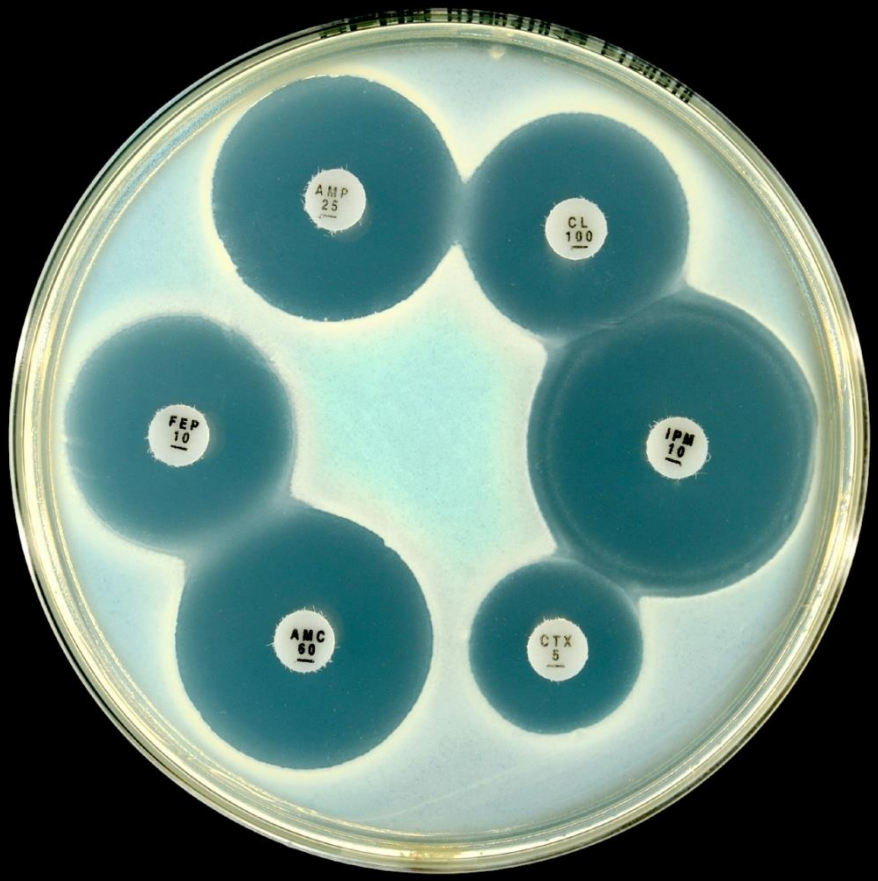
Resistant to ampicillin (AMP 25)

An *E. coli* producing an inhibitor resistant TEM (IRT)
Resistant to ampicillin (AMP 25) and Augmentin (AMC 60)
Susceptible to cephalexin (CL 100), imipenem (IPM 10), cefotaxime (CTX 5).

Confirmation of IRT:

R/ Augmentin (AMC 60), Timentin (TIM 85) and Tazocin (TZP 55)

S/ cephalexin (CL 100), ceftazidime (CAZ 10) and cefotaxime (CTX 5).



A typical *Acinetobacter lwoffi* (β-lactamase negative)

S/ ALL

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

High level of non inducible AmpC

R/ ampicillin (AMP 5), cephalexin (CL 100),
cefotaxime (CTX 5), cefepime (FEP 10), Augmentin (AMC 60)

S/ imipenem (IPM 10)

A strain of *Acinetobacter* sp.(*Acinetobacter* genomospecies 3)

Low AmpC activity

R/ CL 100 (cephalexin), AMP 25 zone > 6 mm with colonies at the edge

Report: R/ ampicillin, cephalexin

Cefotaxime is borderline (6mm) => R/S

Enterobacteriaceae / Acinetobacter sp.

Always test AMP 25 and CL 100 in parallel.
(Cephalexin highly susceptible to AmpC hydrolysis)

1. **S/ AMP 25, CL => S/ Ampicillin, amoxicillin, CL**

2. **R/ AMP 25, CL => R/ Ampicillin, amoxicillin, CL**

3. **R/ AMP; S/ CL 100 => R/ Ampicillin, amoxicillin**
   S/ CL (TEM-1)

4. **S/ AMP; R/ CL 100 => R/ Ampicillin, amoxicillin, CL**
   (Low AmpC)
   (optional: confirm by nitrocefin testing)

ESBLs

(Ambler class A, Bush group 2)

Inhibited by CA

R/ **Cephalosporins including cefepime (FEP) and aztreonam (ATM)**

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 urine isolates

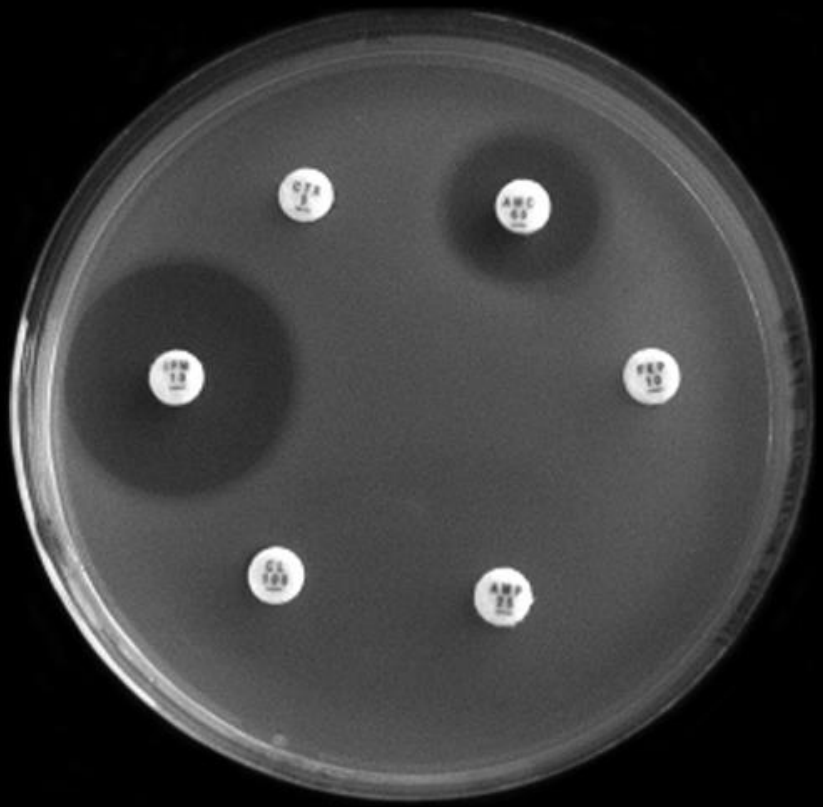
Klebsiella pneumoniae **producing an ESBL in routine CDS test**

S/ Augmentin (AMC 60), typical synergy between Augmentin and cefotaxime (CTX 5) / cefepime (FEP 10)

Report: S/ imipenem (IMP 10)

Disc positions recommended for urine isolates

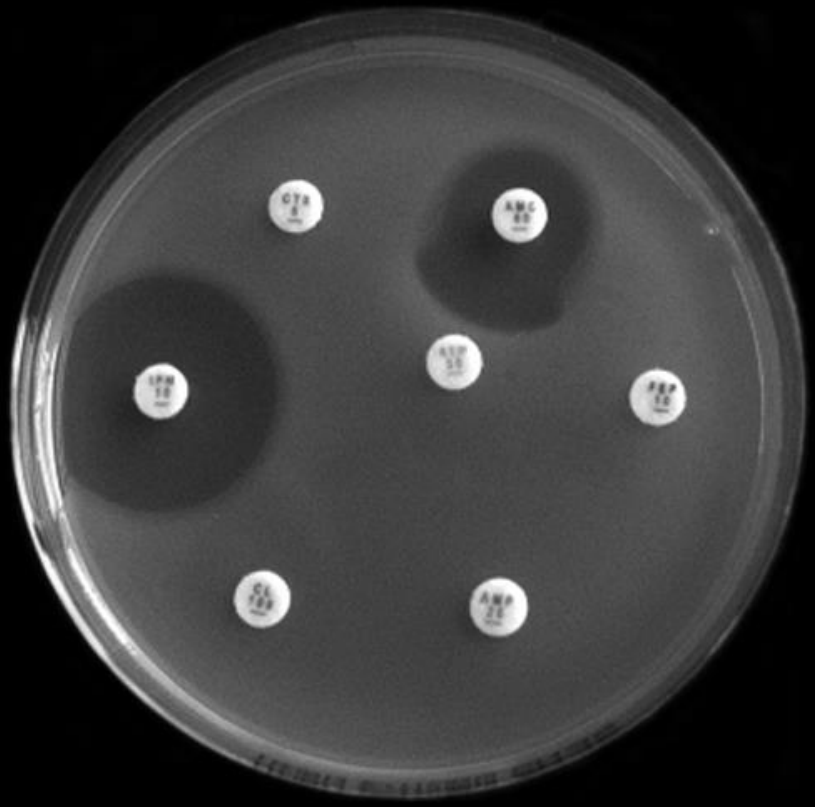
Klebsiella pneumoniae **producing an ESBL: synergy between Augmentin (ACM 60) and cefepime (FEP 10), no obvious synergy with cefotaxime (CTX 5) Probable intrinsic resistance and high ESBL activity.**



Disc positions recommended for urine isolates

***E. coli*: No obvious synergy between Augmentin (AMC, borderline) and cefepime (FEP, 0 mm) or cefotaxime (CTX, 0 mm); probable high ESBL activity of ESBL and intrinsic resistance.**

S/ IMP (large zone, no resistant colonies)



Detection of high activity ESBL or ESBL + intrinsic resistance

Same *E. coli*: synergy between Augmentin (AMC) and aztreonam (ATM) placed in the centre, close to AMC disc.

Need ATM to show synergy with AMC 60=> ESBL

PM-AmpC in *E. coli/ Klebsiella*

R/ AMC 60 (not inhibited by CA)

R/ CL 100

S/ FEP 10

Standard interpretation

(No resistant mutants)

> 6 mm (no resistant col.) => S/ CTX 5

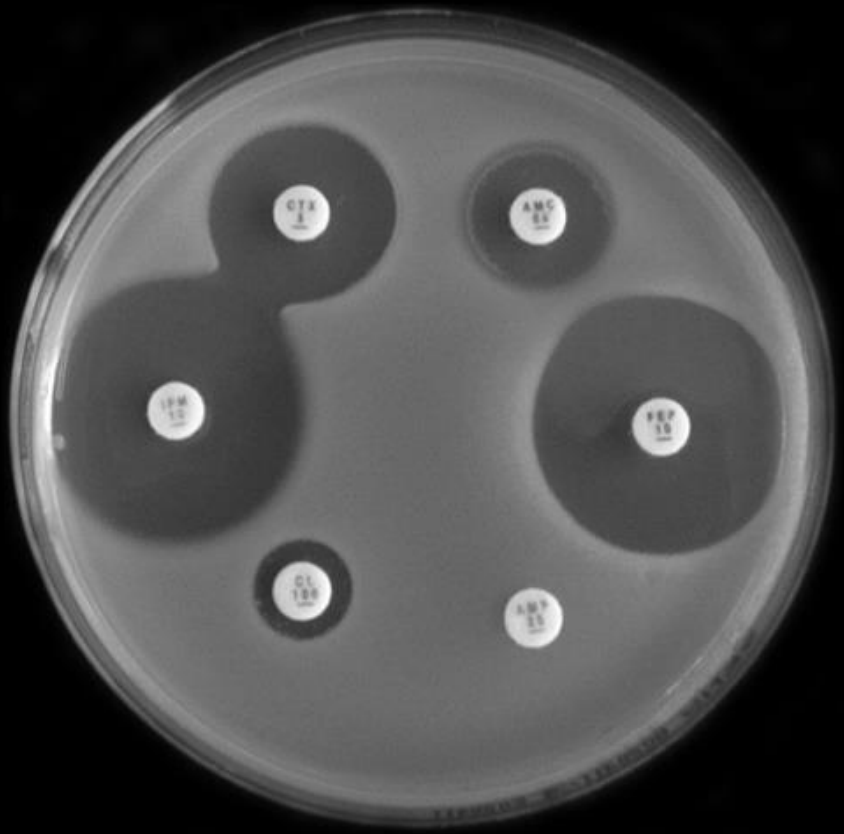
< 6 mm => R/ CTX 5

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

Routine CDS test showing an *E. coli* with a TEM-1 and a plasmid mediated AmpC
R/ ampicillin (AMP) Augmentin (AMC), cephalexin (CL), cefotaxime (CTX)
S/ cefepime (FEP 10) and imipenem (IPM 10).

The same *E. coli* (PM-AmpC)
Synergy between boronic acid discs (blank) and adjacent discs: cefotaxime (CTX 5), Augmentin (AMC 60), cephalexin (CL 100), ceftazidime (CAZ 10).
Blank disc= 250 µg boronic acid disc

S/ FEP, IMP



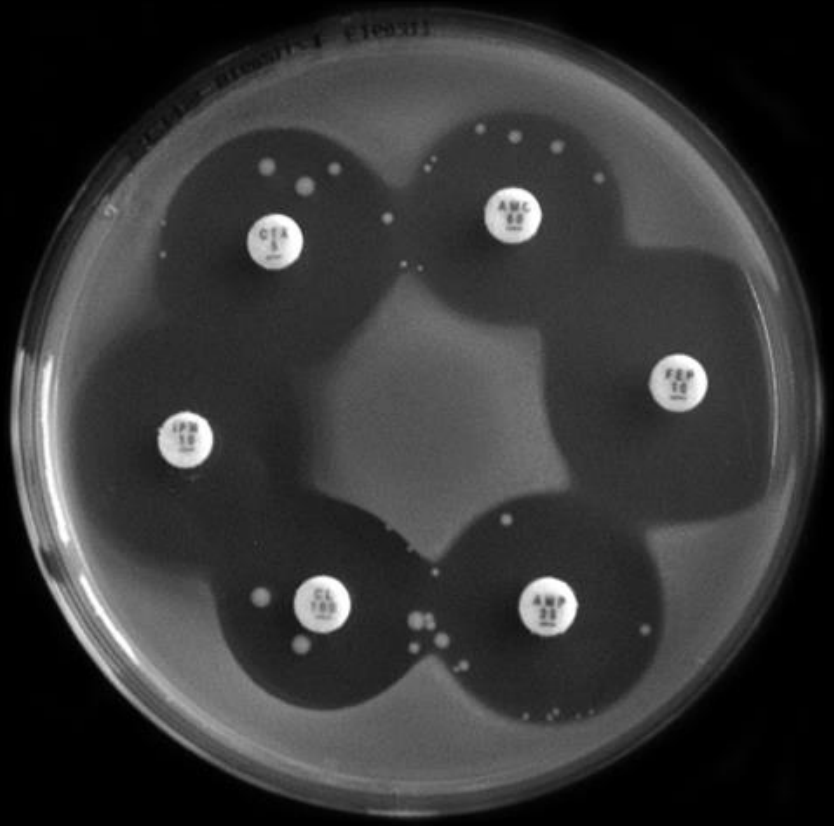
Routine CDS test showing an *E. coli* with a TEM-1 and a LOW ACTIVITY plasmid mediated AmpC: AMP (0 mm), AMC (5 mm), CL (2 mm), CTX (7.5 mm), FEP (12 mm), IMP (12 mm). No resistant mutants in CTX zone.

Report: **R/ AMP, AMC, CL**

S/ CTX, FEP, IMP

Routine CDS test showing an *E. coli* with an inducible PM AmpC/resistant mutants (very rare):
R/ ampicillin (AMP), Augmentin (AMC 60), cephalexin (CL 100), cefotaxime (CTX 5) + resistant colonies in the zone.

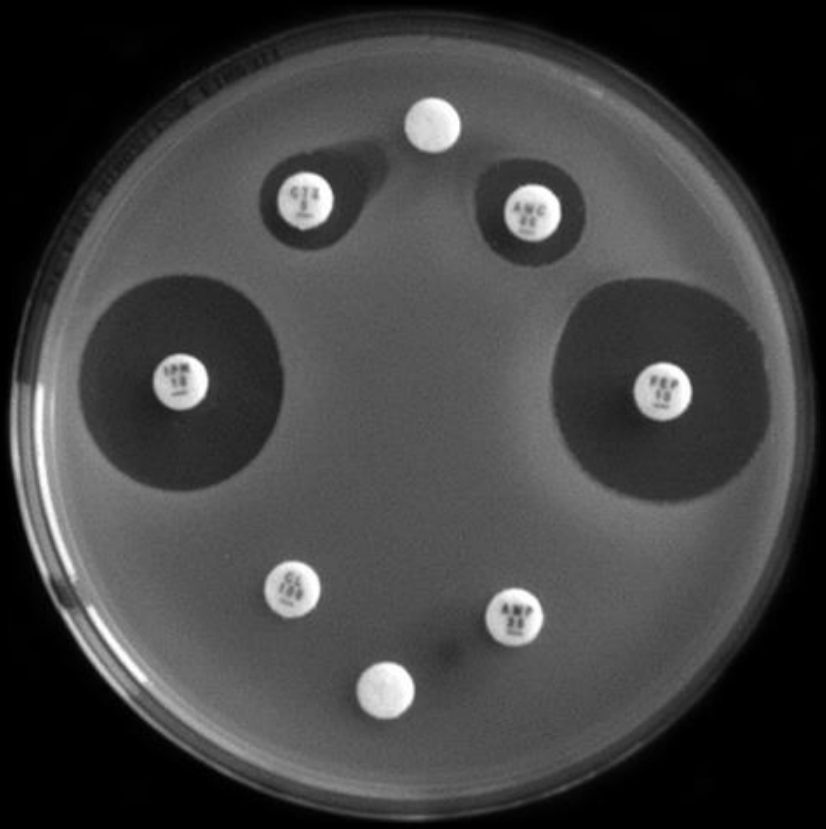
Report: **S/ cefepime (FEP 10) and imipenem (IPM 10)**



CDS test showing an *E. coli* with an unusual PM AmpC from B/C

Large zones around ampicillin (AMP 25, no TEM-1), Augmentin (AMC 60), cephalexin (CL 100), cefotaxime (CTX 5) + resistant colonies in the zones. S/ cefepime (FEP 10) and imipenem (IPM 10) with clear zone, no colonies.

Report: S/ cefepime, imipenem



Same *E. coli:* resistant colony in CTX zone

R/ ampicillin (AMP), Augmentin (AMC 60), cephalexin (CL 100), cefotaxime (CTX 5) showing synergy with boronic acid (blank disc).

Report: S/ cefepime, imipenem

Acquired Metallo-Beta-Lactamases (MBLs)

Ambler class B or Bush group 3

* **Inhibited by EDTA (Zinc molecule)**

  IMP-4 (most common)

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

  NDM (new)

* **Hydrolyses all beta-lactam (except aztreonam)**

  Enterobacteriaceae

    May have a zone > 6mm with IPM 10

  Pseudomonas aeruginosa

    Highly resistant to all β-lactams => no zone

    S/ aztreonam

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

Simple phenotypic detection of MBL:

Same isolate showing synergy between an EDTA (blank) discs placed next to cefotaxime (CTX)/ imipenem (IPM)/ cefepime (FEP)/ ertapenem (ETP) discs.

S/ ATM

***Klebsiella pmeumoniae*:**

Synergy between EDTA (blank discs) and IPM 10/ETP 10 only

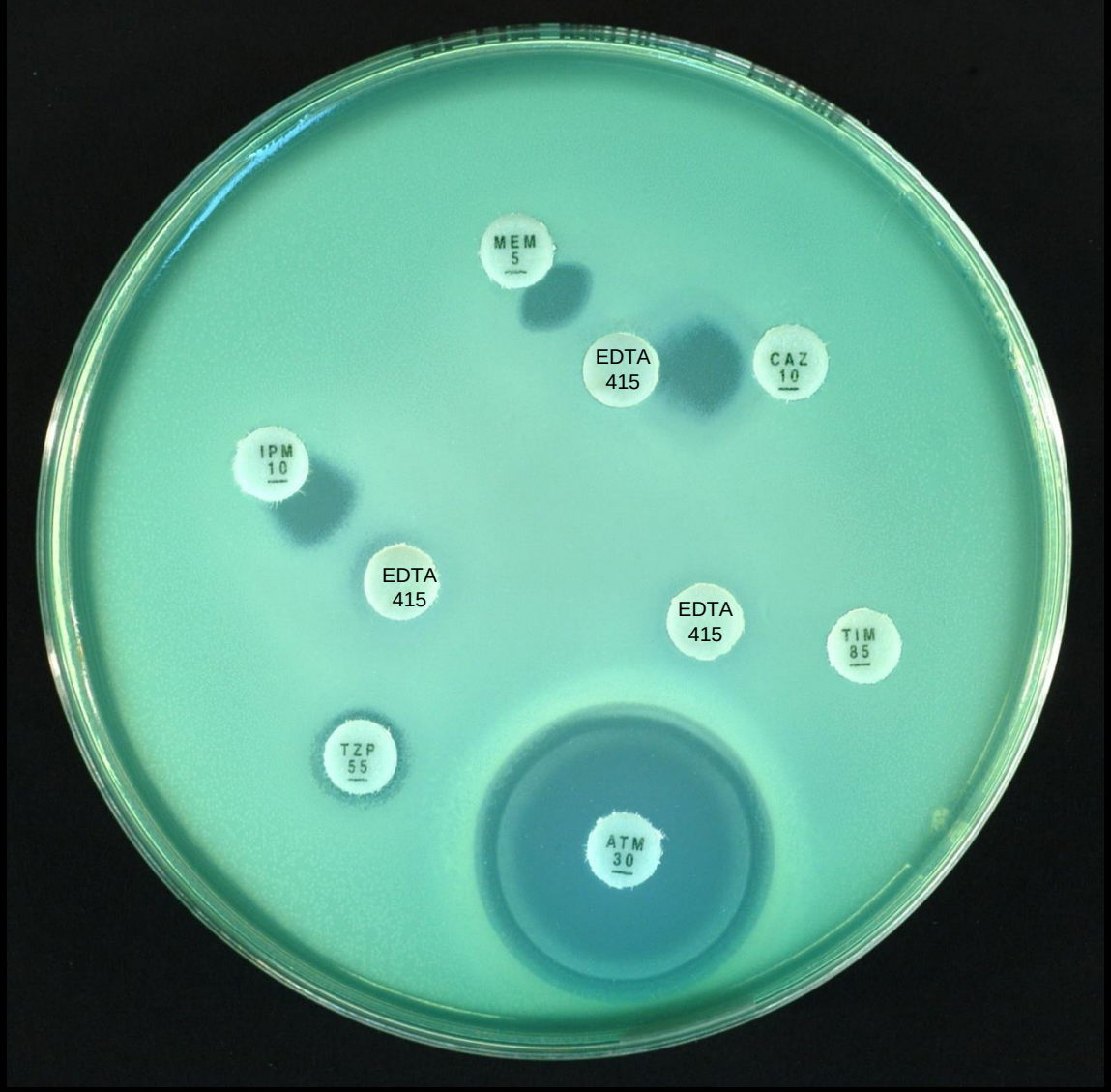
R/ ATM and synergy with AMC 60

=> MBL and ESBL

Pseudomonas aeruginosa **candidate for MBL detection:**

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

S/ aztreonam (ATM 30)



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)

Pseudomonas aeruginosa **resistant to all β-lactams, imipenem (IPM 10) ceftazidime (CAZ 10), tazocin (TZP 55), cefepime (FEP 10) and Timentin (TIM 85) including aztreonam (ATM 30).** **???**

*** Small zone around MEM 5, IPM 10 and ATM 30**

The same *P. aeruginosa*:

Synergy between EDTA and ATM/TZP/CAZ/TIM and MEM 5 but not with IPM => Non specific synergy between EDTA and beta-lactams => not MBL

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

No synergy between EDTA and IPM 10 **???**

The same *K. pneumoniae:*

Synergy between AMC 60 and IPM 10 => inhibited by clavulanate

??? A β-lactamase of Ambler class A or Bush group 2 hydrolysing carbapenem

=> a KPC-2 producing *K. pneumoniae* from Greece

KPC in *Klebsiella pneumoniae*

Plasmid mediated *K. pneumoniae* carbapenemase (KPC)
Ambler class A or Bush group 2f

- **Reported in Europe, US (Brooklyn 24%)**
- **Australia (first isolate in Sept 2010)**
- **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 (border line)**
- **Ertapenem MIC > 8 mg/L (resistant)**
- **Inoculum dependent => broth MIC unreliable**

Summary: "Super" ESBL + R/IPM or colonies at edge of IPM zone
In doubt, test ertapenem and/or send for confirmation

New Table 10.4

A guide to the testing and reporting of β-lactam antibiotics for Gram-negative organisms.

1. **EEC to replace ESCHAPM (Table 10.4)**
2. ***Serratia marcescens* (Table 10.4)**
3. ***Aeromonas* sp. (Table 10.4)**
4. **HPM: standard interpretation**
5. ***K. oxytoca*: standard interpretation**
6. **PM AmpC: standard interpretation**