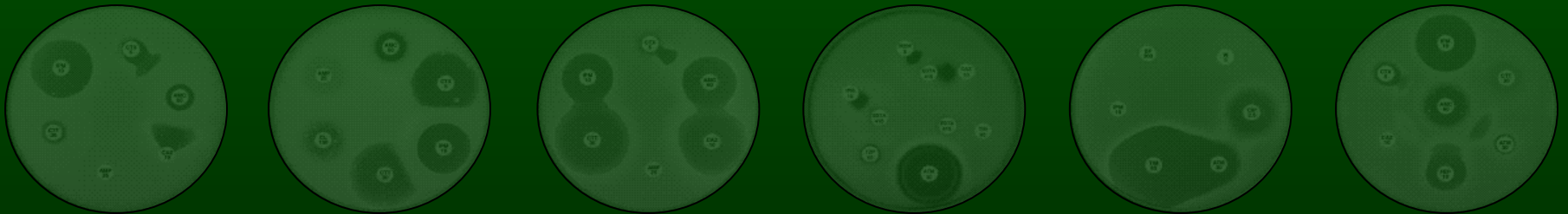


Australian Society of Microbiology
Annual Conference 2008

CDS Workshop



[Start](#)

ASM 2008 – CDS Workshop

- **Opening** (S. M. Bell)
- **CDS website** (G. T. Fisher)
- **Reference Organisms – maintenance of** (G. T. Fisher)
- **β -lactamase of Gram-negative bacilli** (J. Pham)
- **Questions**
- **End**

Tetracycline

- Haemophilus influenzae with efflux

MIC = 32 mg/L = Resistant

30 µg disc annular radius = 5 to 7 mm

10 µg disc annular radius = 3 to 4 mm

- 30 µg disc replaced with a 10 µg disc
- CDS Susceptible/Resistant cut-off point (10 µg disc)

Enterobacteriaceae	}	4 mm
Vibrionaceae		
Acinetobacter		

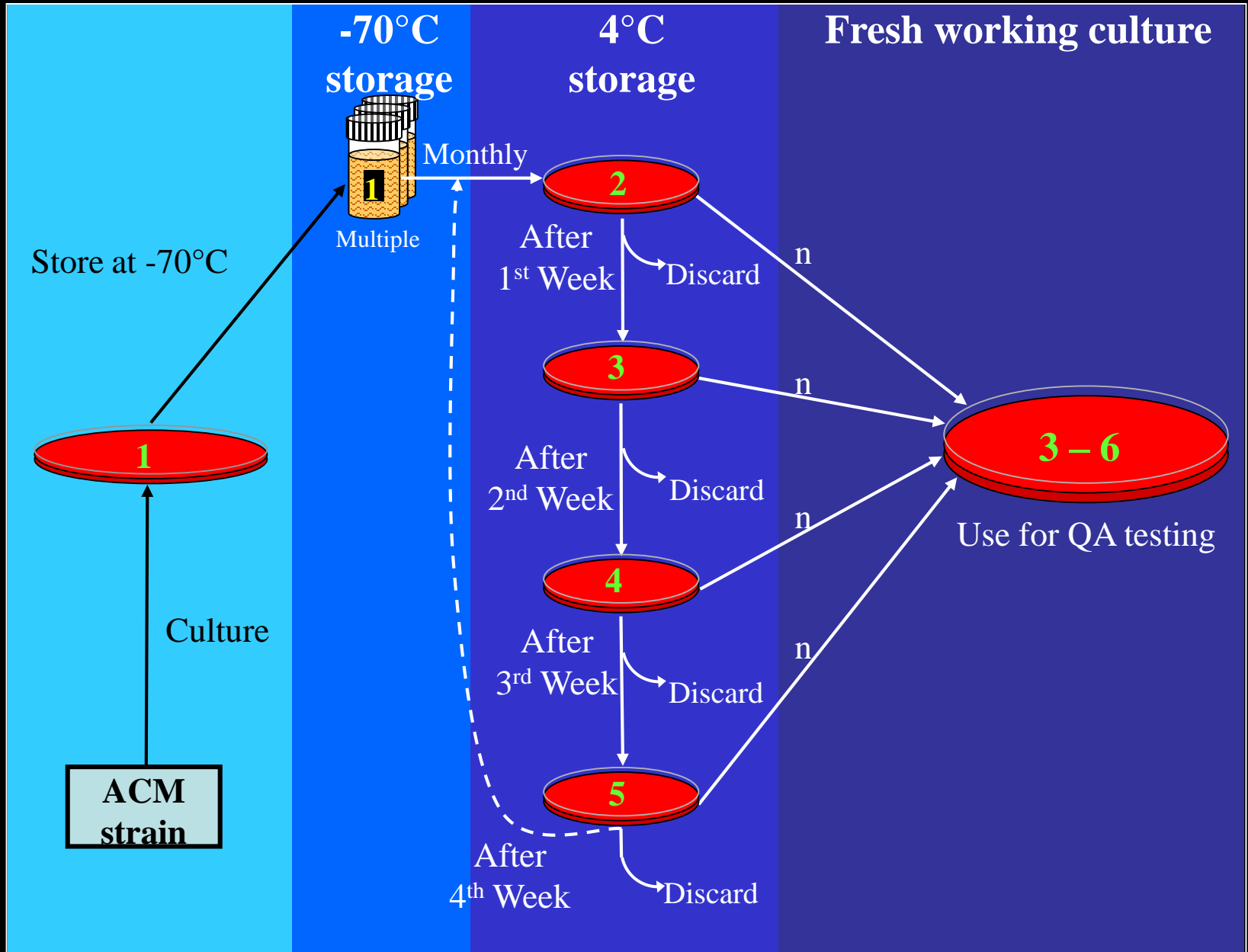
All other organisms	6 mm
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Storage and Recovery of Reference Organisms

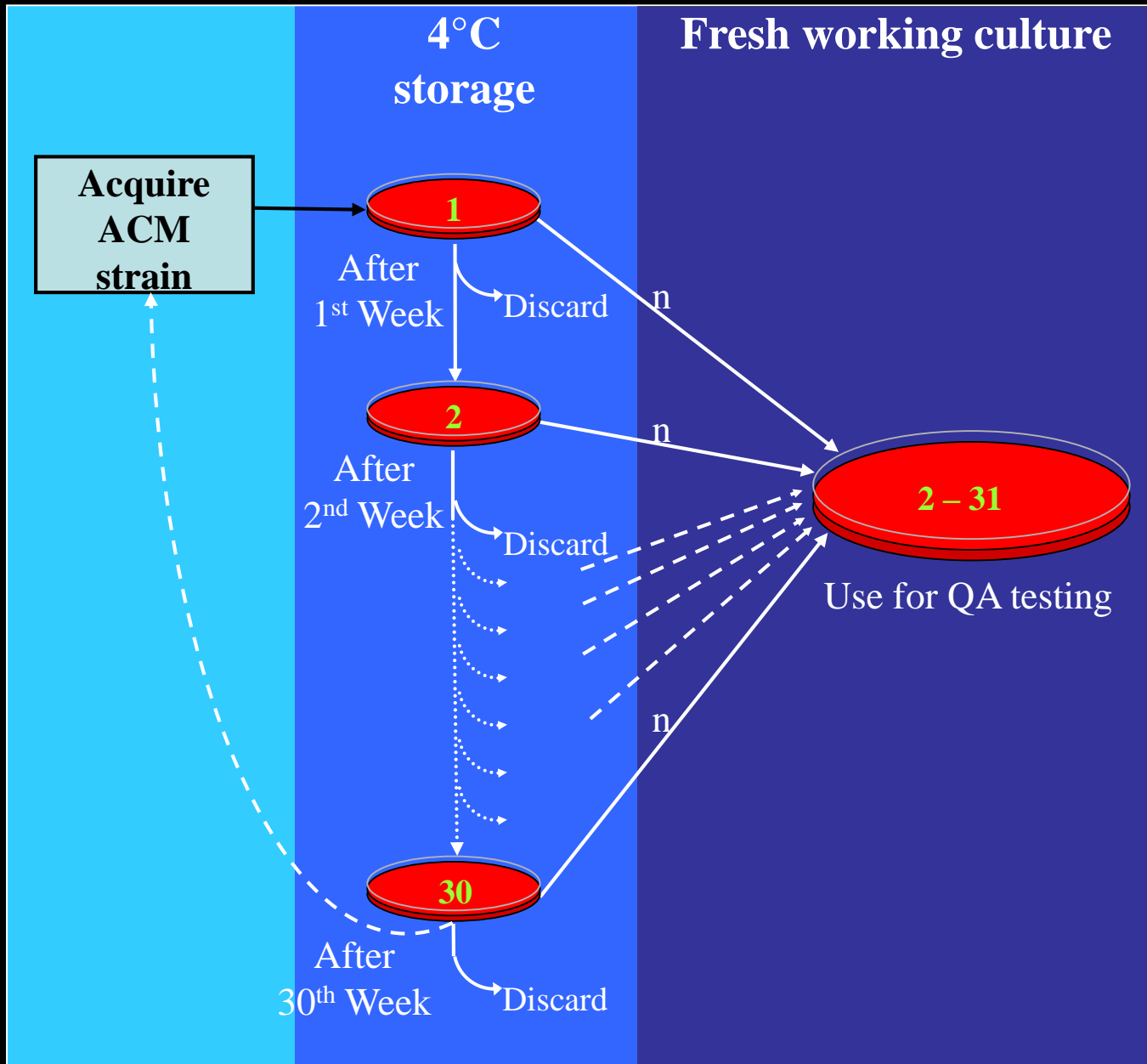
Reference Organism Reliability

- Minimise genetic change by
 - Minimising subculturing
 - Suspending or reducing metabolism

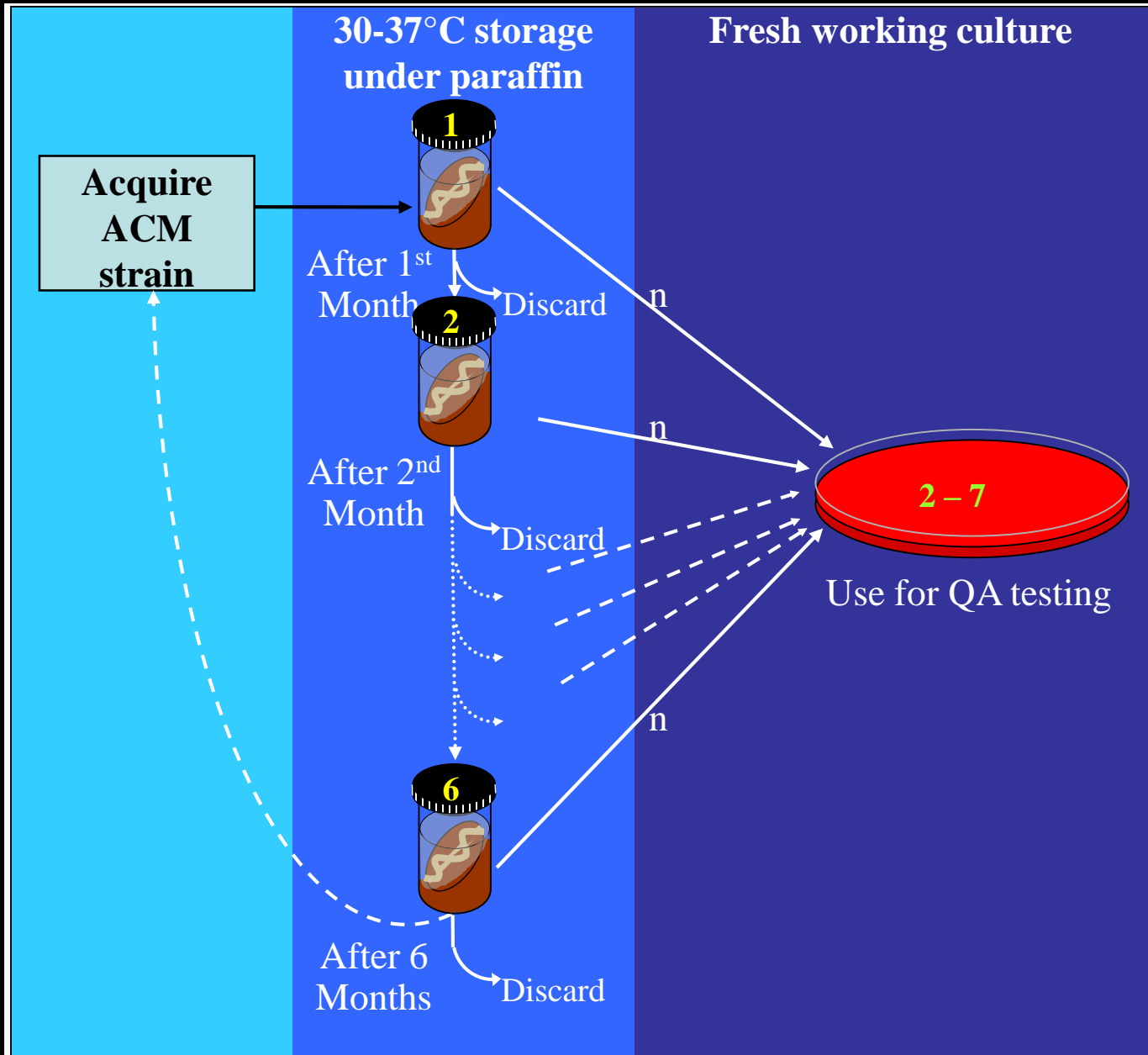
-70°C Storage and Recovery of Reference Cultures



4°C Storage and Recovery of Reference Cultures



Storage and Recovery of *Neisseria gonorrhoeae*



	CDS	ATCC	USP	CLSI
Passages	6 QA testing	5 standard protocols	5 when referring to ATCC number	
2 to 8°C	1 week	1 week		1 to 2 weeks
-20°C		< 1 month		Prolonged
-50 to -70°C			✓	1 year
-70°C	Long term		✓	1 year
< -70°C	Long term		✓	Indefinitely
-80 °C	Long term	Long term	✓	Indefinitely
Liquid Nitrogen Immersion	Long term	✗	✓	Indefinitely
Liquid Nitrogen Vapour Phase	Long term	Long term	✓	Indefinitely

The β -lactamases of Gram-negative bacilli

An update
on detection of **common** plasmid
mediated β -lactamases in clinical
isolates in **Australia**

Classification of β -lactamases

Ambler class

(molecular structure)

A (eg. TEM, ESBL)

B (MBL)

C (AmpC)

D (OXA)

Bush group

(inhibitor)

Group 2

(inhibited by CA)

Group 3

(not inhibited by CA
inhibited by EDTA)

Group 1

(not inhibited by CA,
inhibited by boronic acid)

Group 4 (A. baumannii)

Common transferable (plasmid mediated) β -lactamases in coliforms

- **TEM-1, ESBLs** (Bush group 2, Ambler class A)
 - S/ AMC 60** → synergy (key hole)
 - Inhibited by CA**
- **AmpC:** (Bush group 1, Ambler class C)
 - R/ AMC 60** **S/ FEP 10**
 - Not inhibited by CA**, inhibited by boronic acid
- **MBL:** (Bush group 3, Ambler class B)
 - R/ AMC 60** **R/ FEP 10**
 - Not inhibited by CA**, inhibited by **EDTA**



E. coli ACM 5186 producing TEM-1 resistant to ampicillin (AMP 25)

ESBLs

(Ambler class A, Bush group 2)

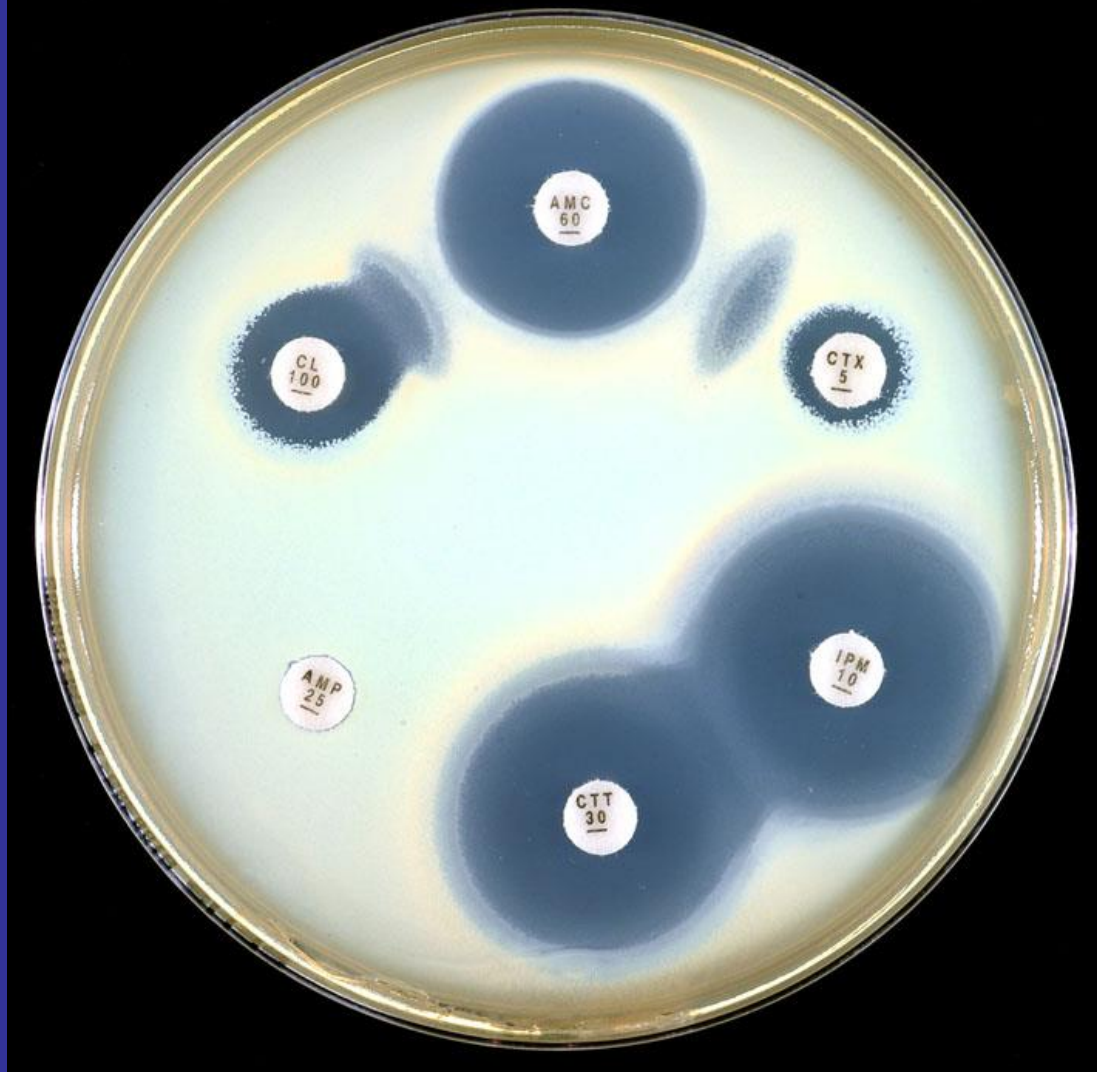
Inhibited by CA

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

**S/ Augmentin (AMC 60)
Cephamycin (cefoxitin, cefotetan)**

**CDS routine testing → Synergy with AMC 60
(no need for confirmation)**

S/ Imipenem (T)



K. pneumoniae producing an ESBL

S/ Augmentin (AMC 60), typical synergy with cephalexin (CL 100) and cefotaxime (CTX 5)

S/ imipenem (IMP 10) and cefotetan (CTT 30).



CDS Routine Testing: Positioning strongly recommended

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



Routine CDS test pattern of an organism of the ESCHAPPM group with an inducible AmpC (flattened CTX 5 zone near IPM, 8 mm + resistant colonies) R/ Augmentin (AMC 60), cephalixin (CL 100), S/ cefepime (FEP 10) and imipenem (IPM 10).

Plasmid mediated AmpC (PM-AmpC)

- R/ clavulanic acid
- Constitutive (no induction, rare exception)
- Similar profile as ESCHAPPM with minor variation
 - R/ 3rd generation cephalosporins, cephamycin (CMY)
 - S/ cefepime (4th generation)
- CMY-1 (1989) in *K. pneumoniae* → *E. coli*
- MIR-1 (1990) in *K. pneumoniae* → *E. coli*
90% identical to *ampC* gene in *E. cloacae*
- Coudron et al (1995-97) examined > 1000 isolates (VA)
Found PM-AmpC in 1.6 % *E. coli* and 1.1% *K. pneumoniae*
- Confusing nomenclature:
 - CMY → resistance to cephamycin (CMY-1.....CMY-21)
 - DHA → Dharhan Hospital in Saudi Arabia
 - ACT → Ambler Class C Type
 - FOX, MOX, LAT → resistance to cefoxitin, moxalactam, latamoxef
(LAT-3 = CMY-6....)
- CMY-2 found in several serotypes of ceftriaxone resistant *Salmonella*

Loss of porin may add to resistance

Detection of PM-AmpC in *E. coli* and *Klebsiella* (non-ESCHAPPM)

R/ AMC 60 (not inhibited by CA)

R/ CL 100

R/ CTX 5 (high level resistance)

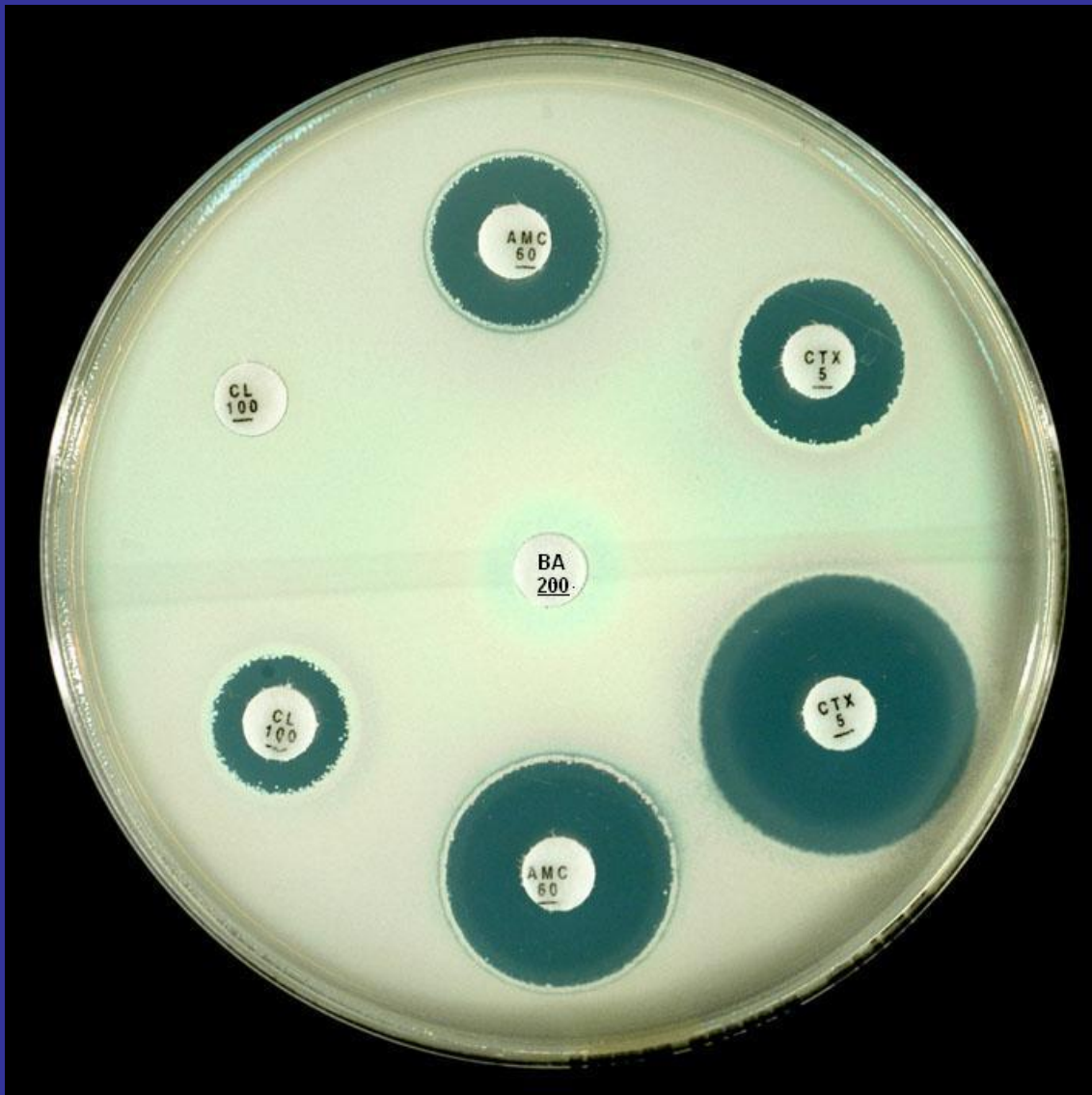
S/ FEP 10

Confirmation (optional): inhibition by boronic acid

1-Benzothiophene-2-boronic acid



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



E. coli with a plasmid mediated AmpC β -lactamase and boronic acid (BA)

Top half: CL 100, AMC 60 and CTX 5 control discs.

Bottom half: CL 100, AMC 60 and CTX 5 with 200 ug BA added



E. coli with a plasmid mediated AmpC β -lactamase (low activity)

Top half: CL 100, AMC 60 and CTX 5 control discs.

Bottom half: CL 100, AMC 60 and CTX 5 with 200 μ g BA added



E. coli with a plasmid mediated AmpC β -lactamase and boronic acid (BA)
Top half: CL 100, AMC 60 and FOX 30 control discs.
Bottom half: CL 100, AMC 60 and FOX 30 with 200 ug BA added
CL 100 used in routine CDS testing is more sensitive to AmpC than FOX 30



E. coli with a plasmid mediated AmpC β -lactamase.
CL 100, AMC 60 and CTX 5 discs placed near 200 ug BA discs

Summary

Recognition of AmpC in routine testing

Criteria to recognise of AmpC in non-ESCHAPPM organism (eg. *E. coli*, *Klebsiella*, *Proteus mirabilis*, *Salmonella*) in routine testing:

CL 100 and ACM 60 < 6 mm

No “key hole” (no synergy with AMC 60)

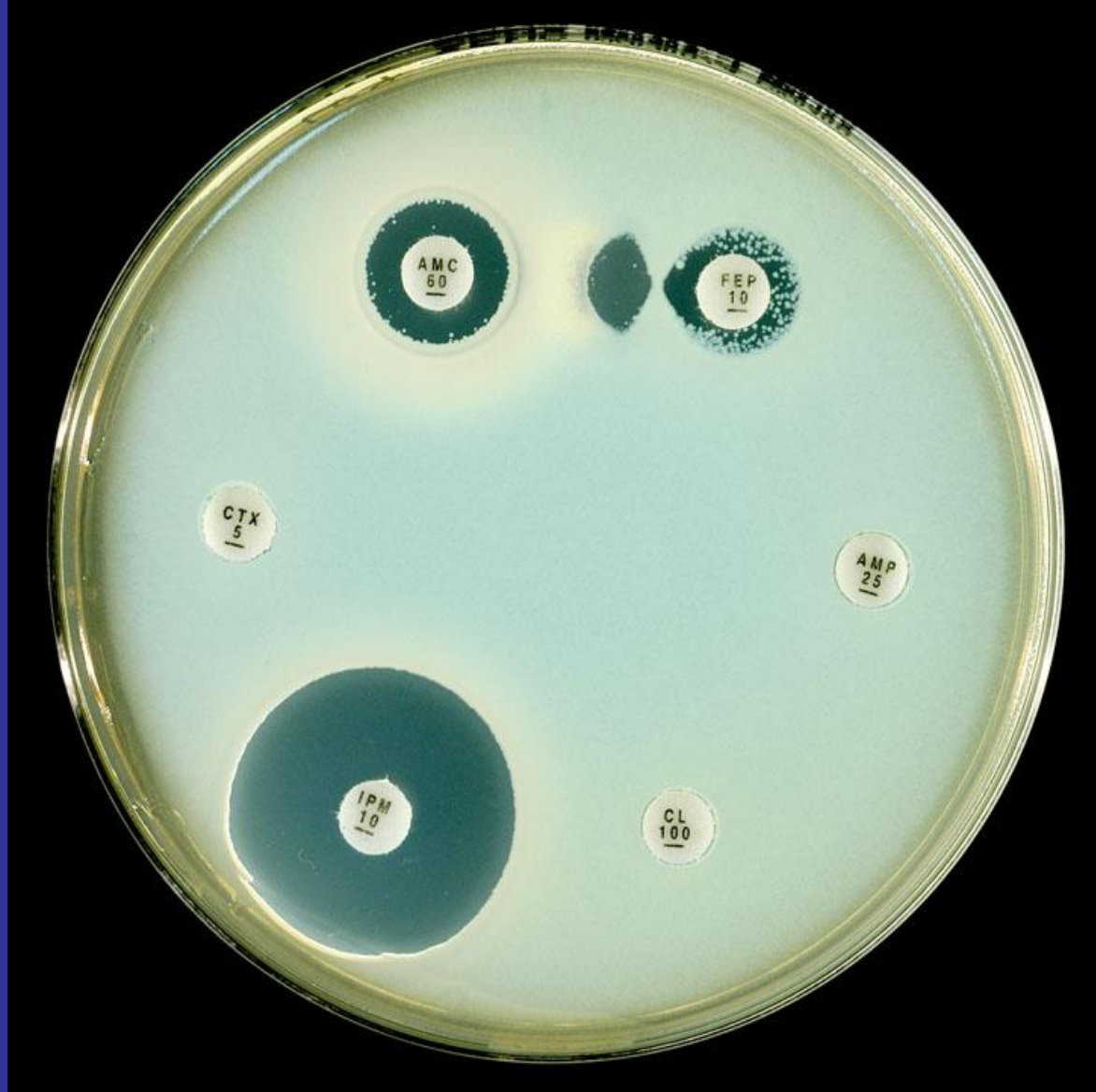
→ AmpC

CTX 5 > 6mm = low level activity

CTX 5 < 6mm or no zone = high level activity

Record in comments section (suppressed)

The organism is usually susceptible to cefepime (FEP 10) unless an ESBL or MBL is present.



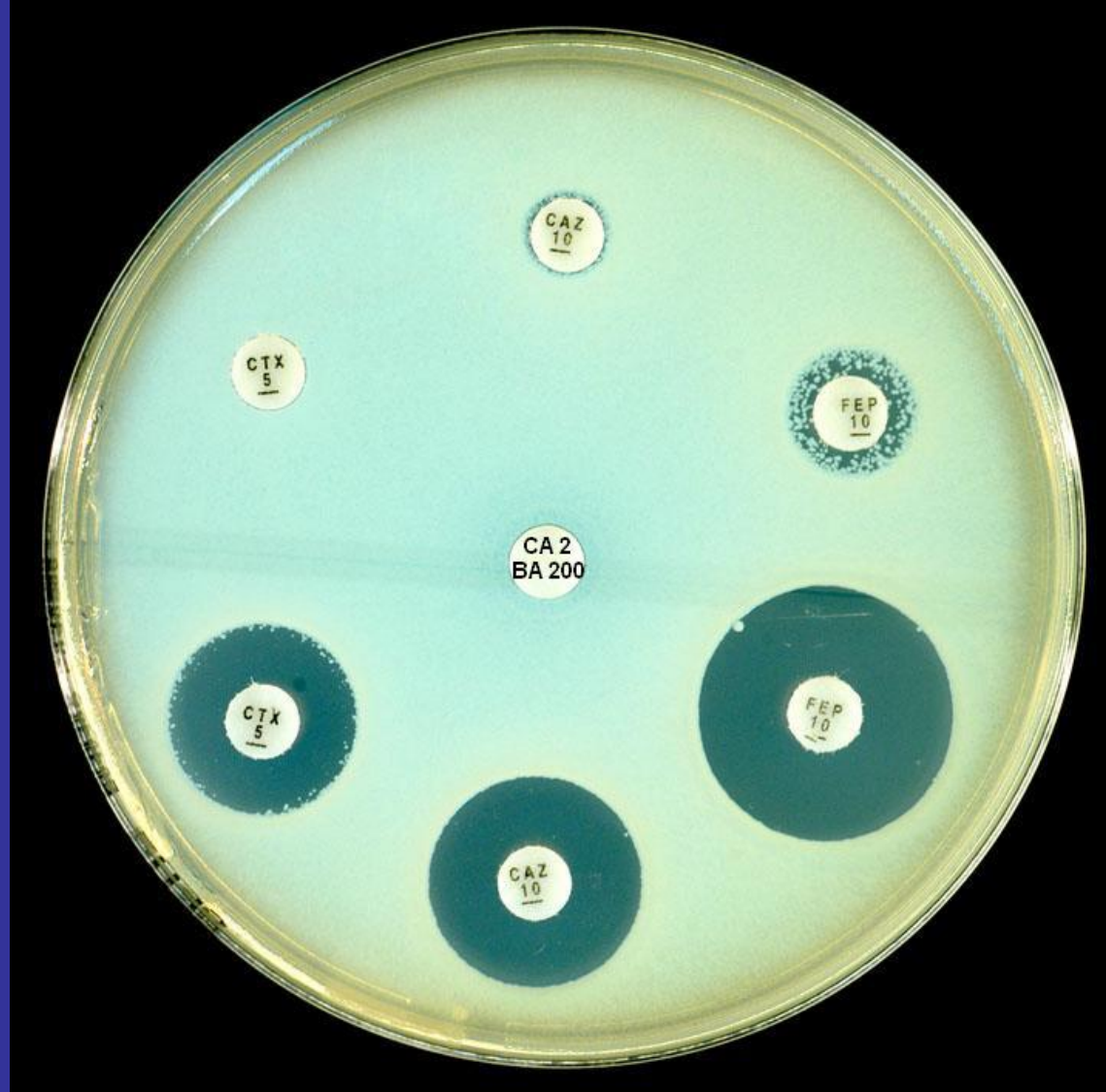
***E. coli* with a PM-AmpC and an ESBL in routine CDS test:
R/ AMC 60 and FEP 10 (and CTX, CL, AMP), synergy between AMC 60 and
cefepime (FEP 10).
S/imipenem (IPM 10)**



The same *E. coli* with a PM-AmpC and ESBL

Top half: CTX 5, CAZ 10 and FEP 10 control discs.

Bottom half: CTX 5, CAZ 10 and FEP 10 with 2 ug clavulanic acid added



E. coli with a plasmid mediated AmpC and ESBL

Top half: CTX 5, CAZ 10 and FEP 10 control discs.

Bottom half: CTX 5, CAZ 10 and FEP 10 with 2 ug clavulanic acid and 200 ug boronic acid.

Classification of beta-lactamases

Ambler class
(molecular structure)

A (eg. TEM, ESBL)

B (MBL)

C (AmpC)

Bush group
(inhibitor)

Group 2
(inhibited by CA)

Group 3
(inhibited by EDTA)

Group 1
(NOT inhibited by
CA or EDTA)

Acquired Metallo-Beta-Lactamases (MBLs)

Plasmid mediated MBLs
Ambler class B or Bush group 3

Inhibited by EDTA (Zinc molecule)

IMP-4 (most common)

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

Hydrolyses all beta-lactam (except aztreonam)

Enterobacteriaceae

May have a zone > 6mm with IPM 10

Pseudomonas aeruginosa

Highly resistant to all β -lactams (S/ATM)



An isolate of *C. diversus* R/ Augmentin (AMC 60), cephalexin (CL100), cefotaxime (CTX 5) with colonies in cefepime (FEP 10) zone and some at the edge of imipenem (IPM 10) zone (> 6 mm).

???



R/AMC 60, CL100, CTX 5 and colonies in cefepime zone (FEP 10) and some at the edge of imipenem zone (> 6 mm).

- **No synergy between FEP/AMC → not ESBL**
- **Numerous resistant colonies in FEP 10 → Candidate for MBL detection**



Simple detection of MBL:

Same isolate showing synergy between an EDTA disc placed next to cefotaxime (CTX 5)/ imipenem (IPM 10)/ cefepime (FEP 10)/ Augmentin (AMC 60) discs.



MBL producing *E. cloacae*:

**Synergy between EDTA and cefotaxime (CTX 5)/ ceftazidime (CAZ 10)/
cefepime (FEP 10) / imipenem (IPM 10).**

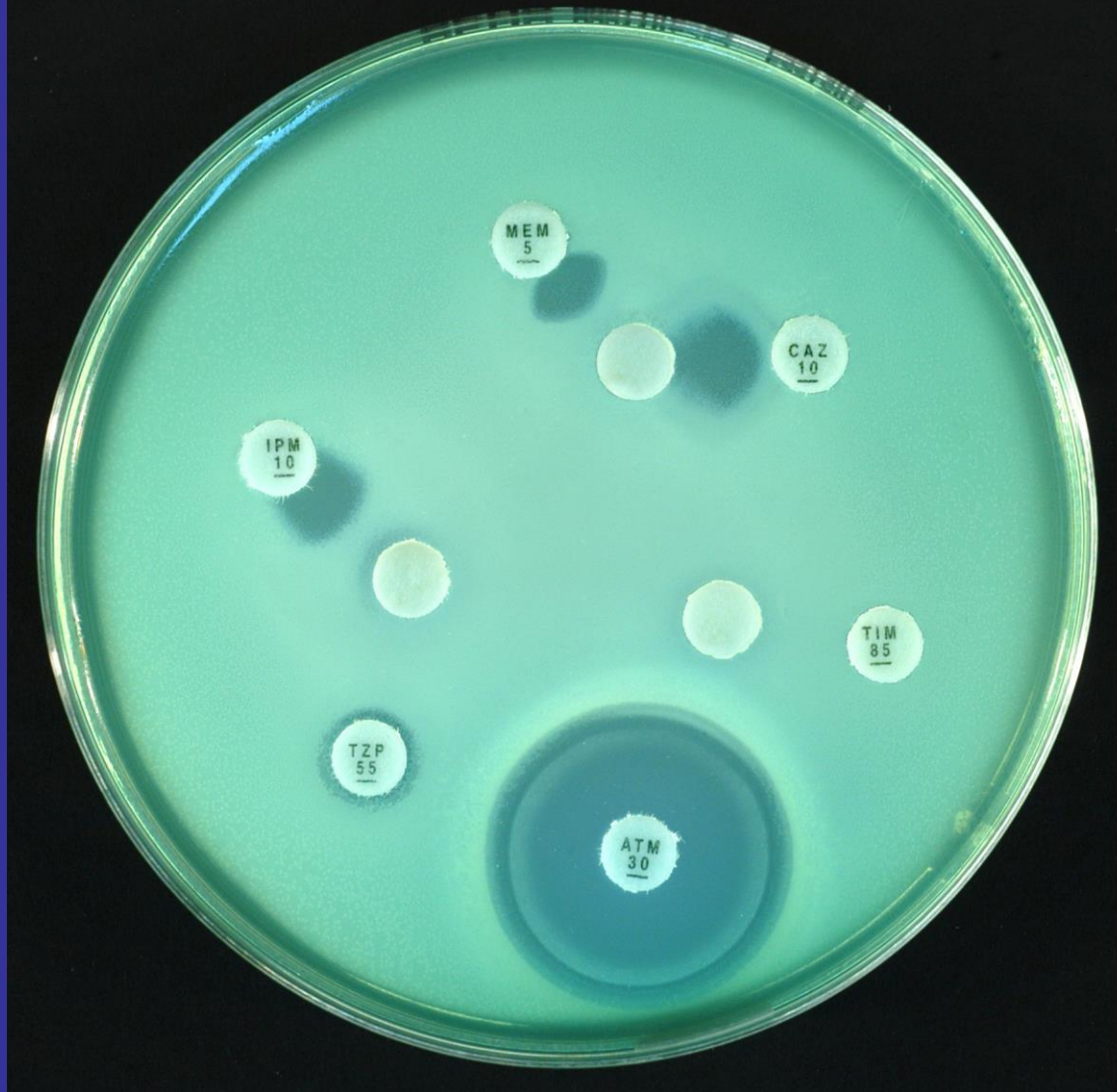
S/ aztreonam (ATM 30)



MBL and ESBL: *K. pneumoniae* showing borderline imipenem zone (IPM 10) and a large zone with IPM 10 loaded with EDTA, synergy between a central EDTA disc and cefotetan (CTT 30). The presence of an ESBL is revealed by the resistance to aztreonam (ATM 30) and synergy between ATM 30 and AMC 60.



Pseudomonas aeruginosa candidate for MBL detection: highly resistant to all β -lactams, imipenem (IPM 10) ceftazidime (CAZ 10), tazocin (TZP 55), cefepime (FEP 10) and Timentin (TIM 85) except aztreonam.



**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)**

