

A review and update of the CDS test

**CDS Workshop
ASM 2009 Perth**

Gram-positive

- **Staphylococci**
- **Streptococci**
- **Enterococci**
- **Corynebacterium sp.**
- **Nocardia (new)**

Staphylococci v/s cefoxitin 10/ oxacillin 1

Standard 6 mm cut off

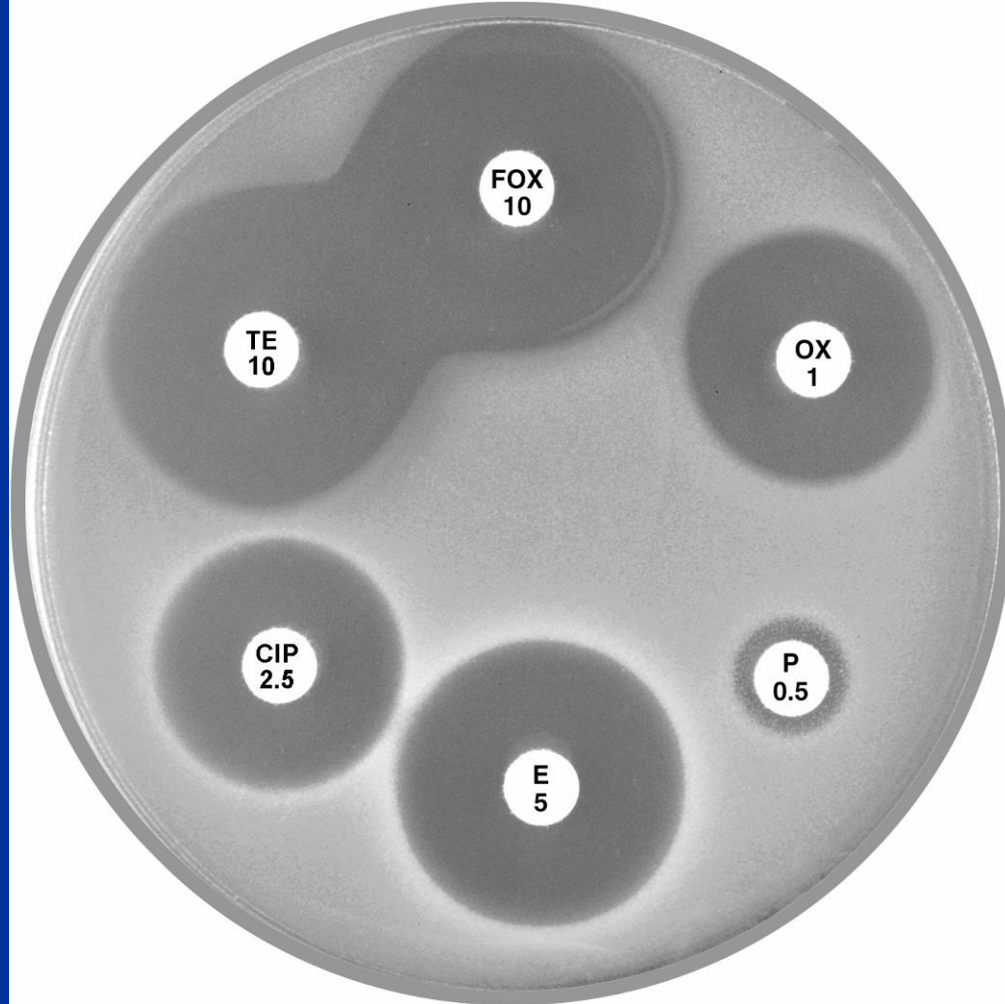
Cefoxitin 10 (Fox 10) for *S. aureus*

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

* No need to incubate at 30°C

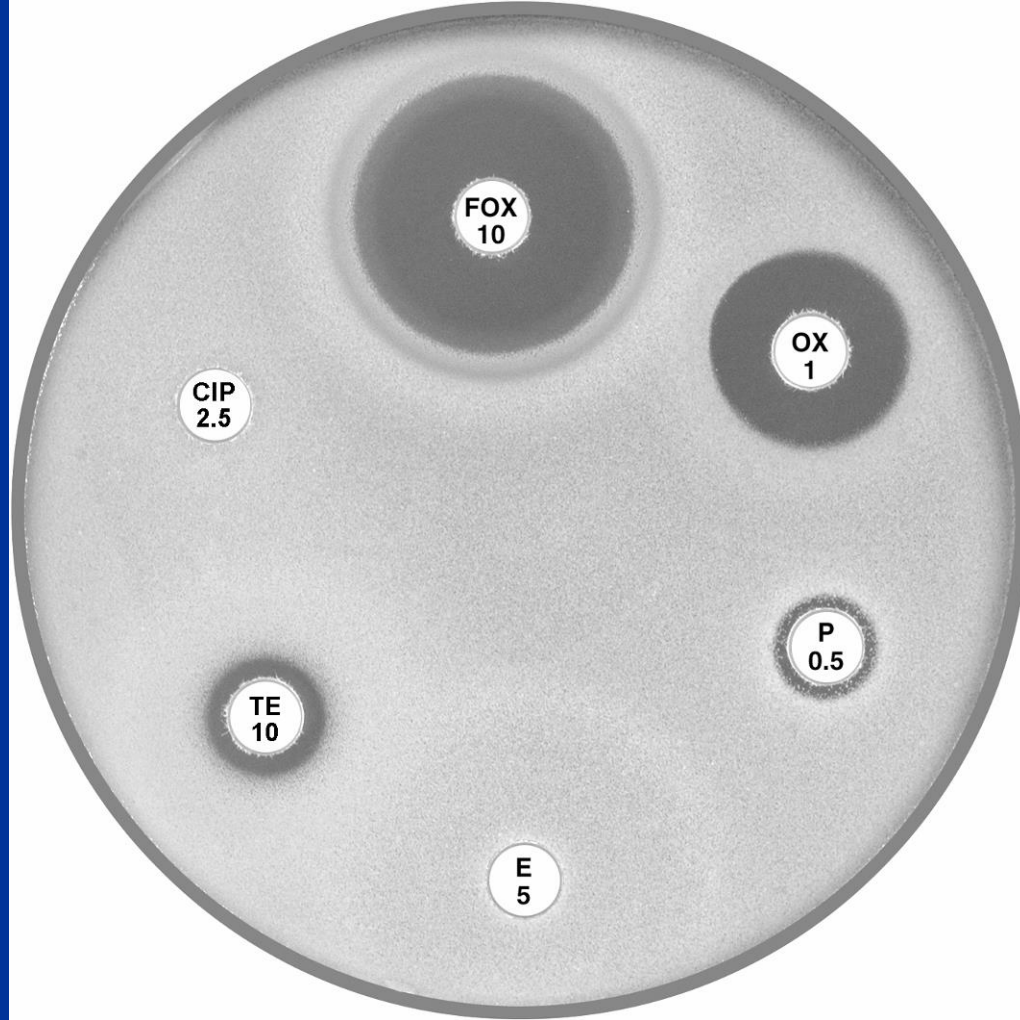
Oxacillin 1 (Ox 1) for CNS

Excellent correlation with *mecA* gene PCR
Report S or R to methicillin



A commonly isolated *S. aureus* resistant to penicillin (P 0.5) and susceptible to cefoxitin (FOX 10) with a zone around 9 mm

Report: S/ MET

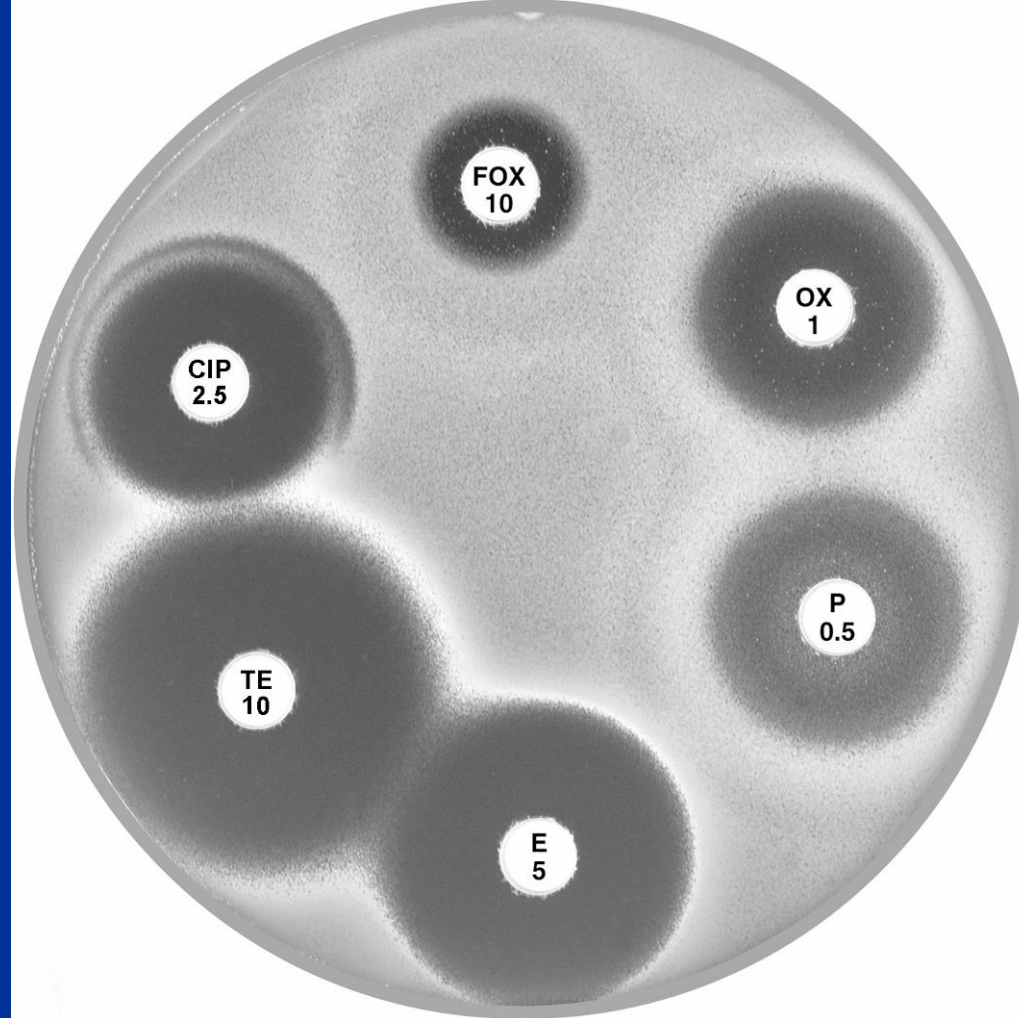


***mecA* gene negative, Multi-Resistant Methicillin Susceptible *S. aureus* (MR-MSSA). Resistant to penicillin (P 0.5), erythromycin (E 5), tetracycline (Te 10) and ciprofloxacin (CIP 2.5) but susceptible to cefoxitin (FOX).**

Report: S/ MET ??? Ex-MRSA



Non multi-resistant MRSA = original CA-MRSA
resistant to penicillin (P 0.5) and cefoxitin (FOX 10) only,
susceptible to erythromycin (E 5), tetracycline (TE 10),
co-trimoxazole (SXT 25) and ciprofloxacin (CIP 2.5).



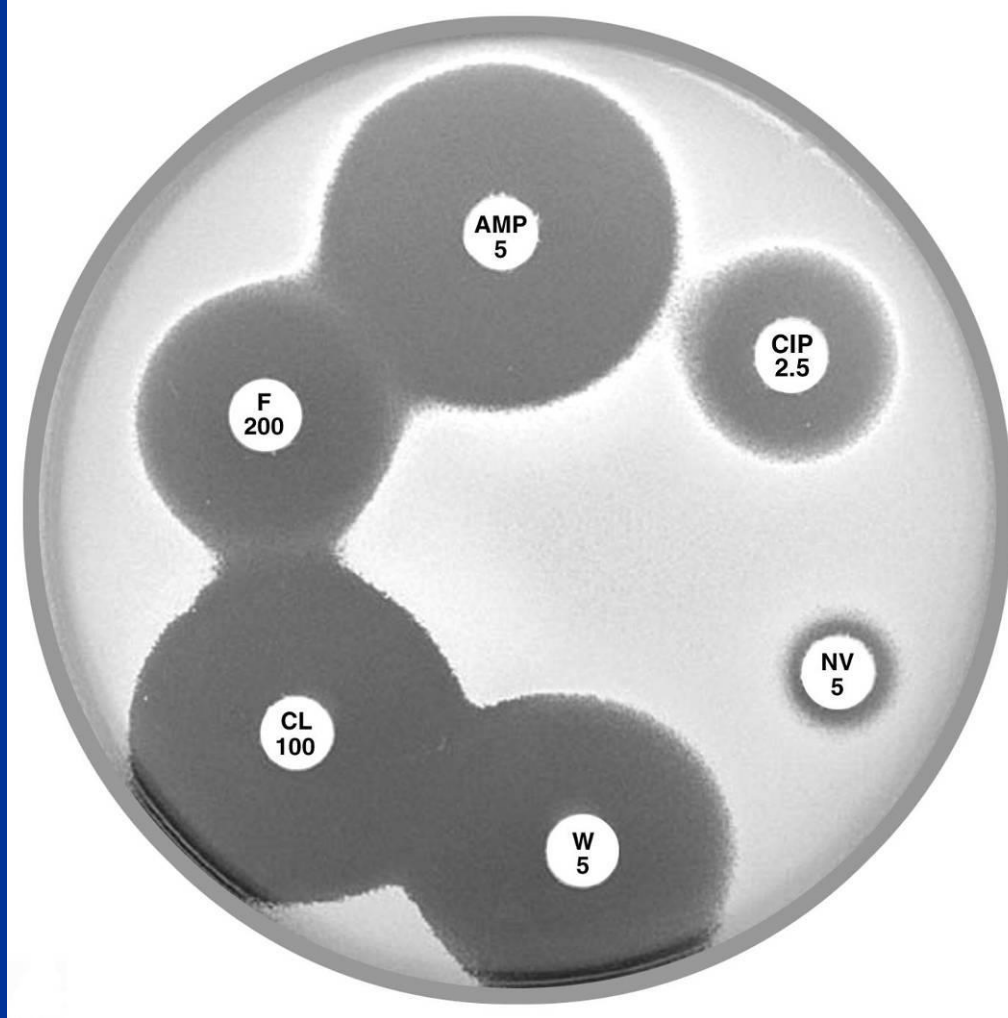
β -lactamase negative NMR- MRSA

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.

S. saprophyticus and β -lactam antibiotics

- UTI isolates of CNS resistant to novobiocin => presumptively identified as *S. saprophyticus*
- Intrinsic resistance => false resistance when
P 0.5 u and Ox 1 are used
 - * Ampicillin 5 μ g (AMP 5) to detect inducible pen'se
 - * Cephalexin 100 μ g (CL 100) to detect *mecA* gene

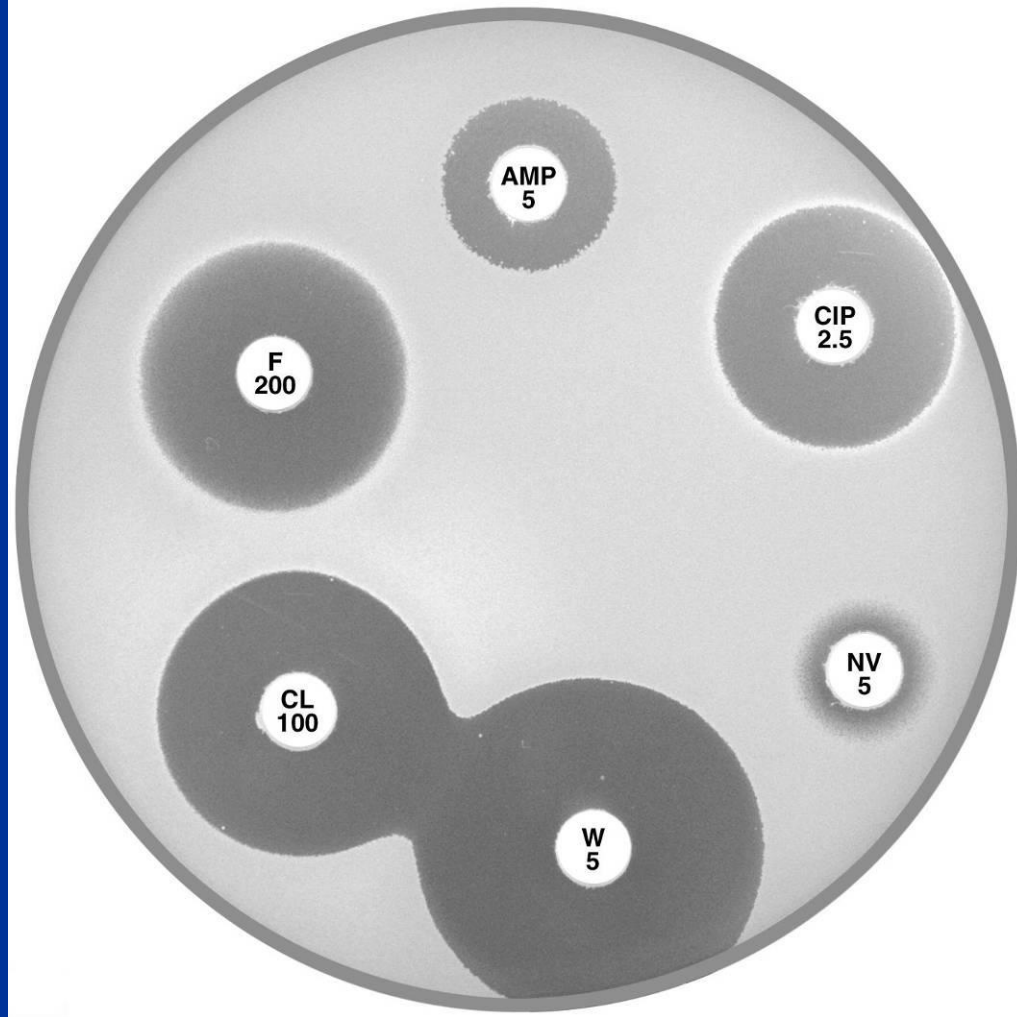


Typical *S. saprophyticus* isolated from UTI

Susceptible to ampicillin (AMP 5) and cephalexin (CL 100).

Also susceptible to trimethoprim (W5), nitrofurantoin (F 200) and ciprofloxacin (CIP 2.5), the surrogate disc for reporting norfloxacin.

Report: S/ penicillin, amoxycillin

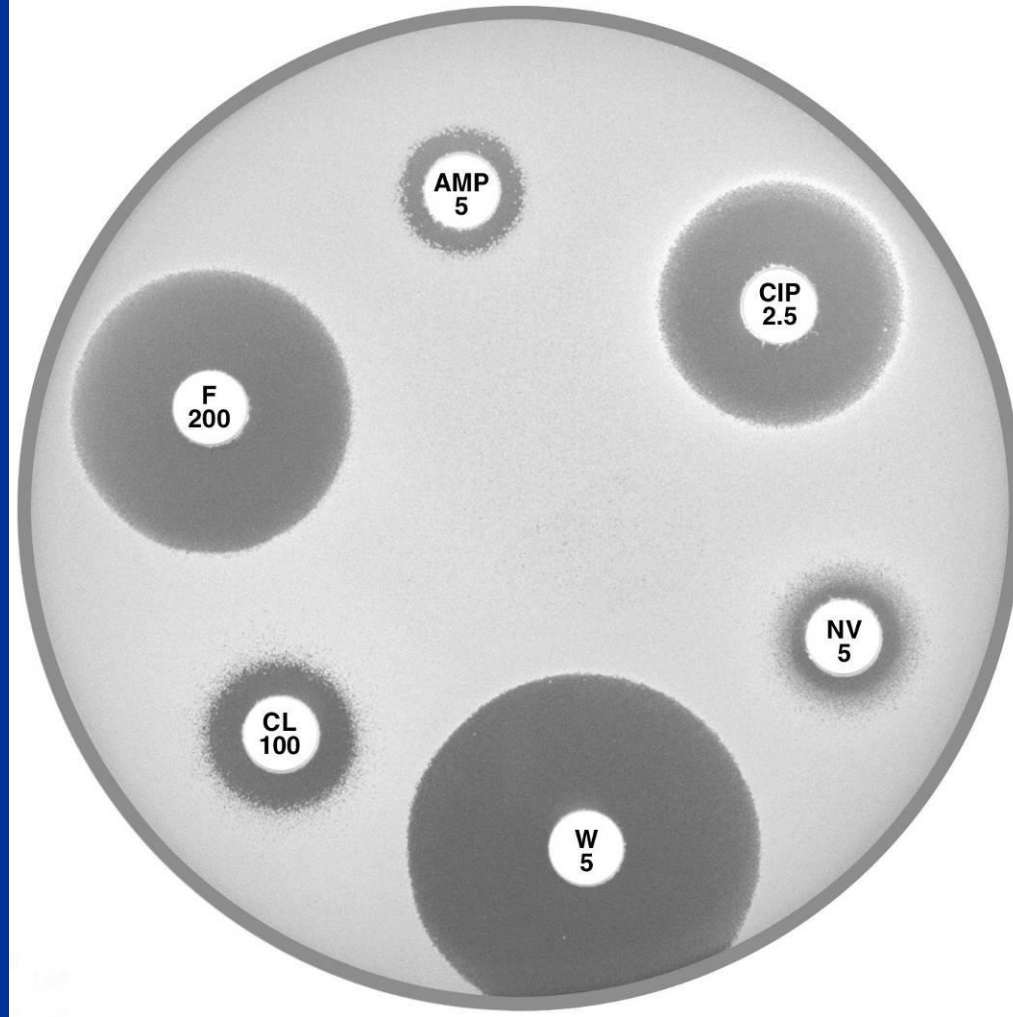


***S. saprophyticus* with an inducible pen'se**

Resistant to ampicillin (AMP 5), susceptible to cephalexin (CL 100), trimethoprim (W5), nitrofurantoin (F 200) and ciprofloxacin (CIP 2.5), the surrogate disc for reporting norfloxacin.

Report: R/penicillin, amoxycillin

S/ cephalexin, Augmentin



MecA* gene positive *S. saprophyticus

Resistant to both ampicillin (AMP 5) and cephalexin (CL 100), susceptible to trimethoprim (W5), nitrofurantoin (F 200) and ciprofloxacin (CIP 2.5), the surrogate disc for reporting norfloxacin.

Report: R/penicillin, amoxicillin, cephalexin, Augmentin

Streptococci and β -lactams

Tested on blood Sensitest agar in 5% CO₂

Penicillin: P 0.5 u and AMP 5

S/ P 0.5

R/ P 0.5

S/ AMP 5 =>

reduced susceptibility with an MIC
between 0.25 and 2 mg/L

Cephalosporins: CTX/CRO 0.5 and CTX/CRO 5

S/ CTX 0.5

R/ CTX 0.5

S/ CTX 5 =>

reduced susceptibility with an MIC
between 0.5 and 2 mg/L

S. pneumoniae from CSF: P 0.5 u and CTX/CRO 0.5

S/ P 0.5

P 0.5 \leq 6mm => R MIC \geq 0.25 mg/L

S/ CTX 0.5

CTX 0.5 \leq 6mm => R MIC \geq 0.5 mg/L

Enterococci

Tested on blood Sensitest agar in 5% CO₂

Ampicillin 5 µg

Gentamicin 200 µg

Linezolid 10 µg

Nitrofurantoin 200 µg

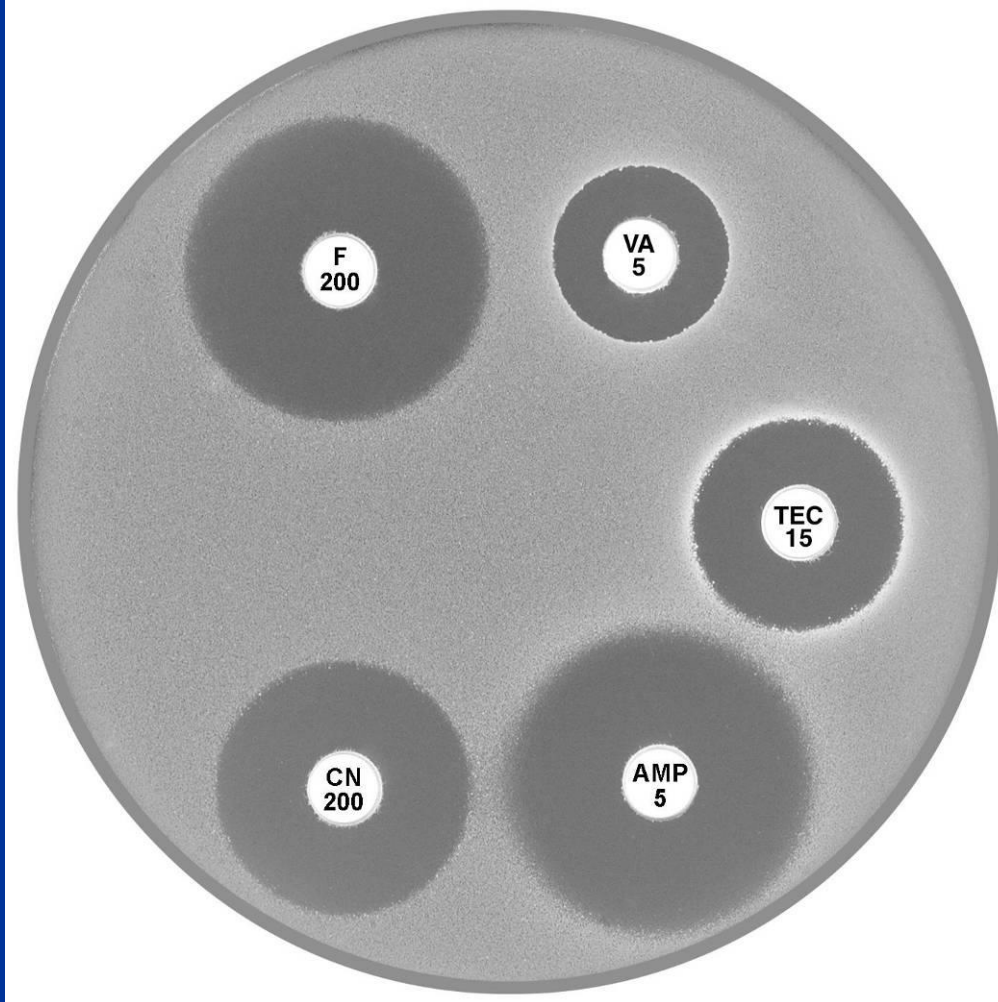
Quinupristin/Dalfopristin 15 µg

Streptomycin 300 µg

Teicoplanin 15 µg

Tigecycline 15 µg

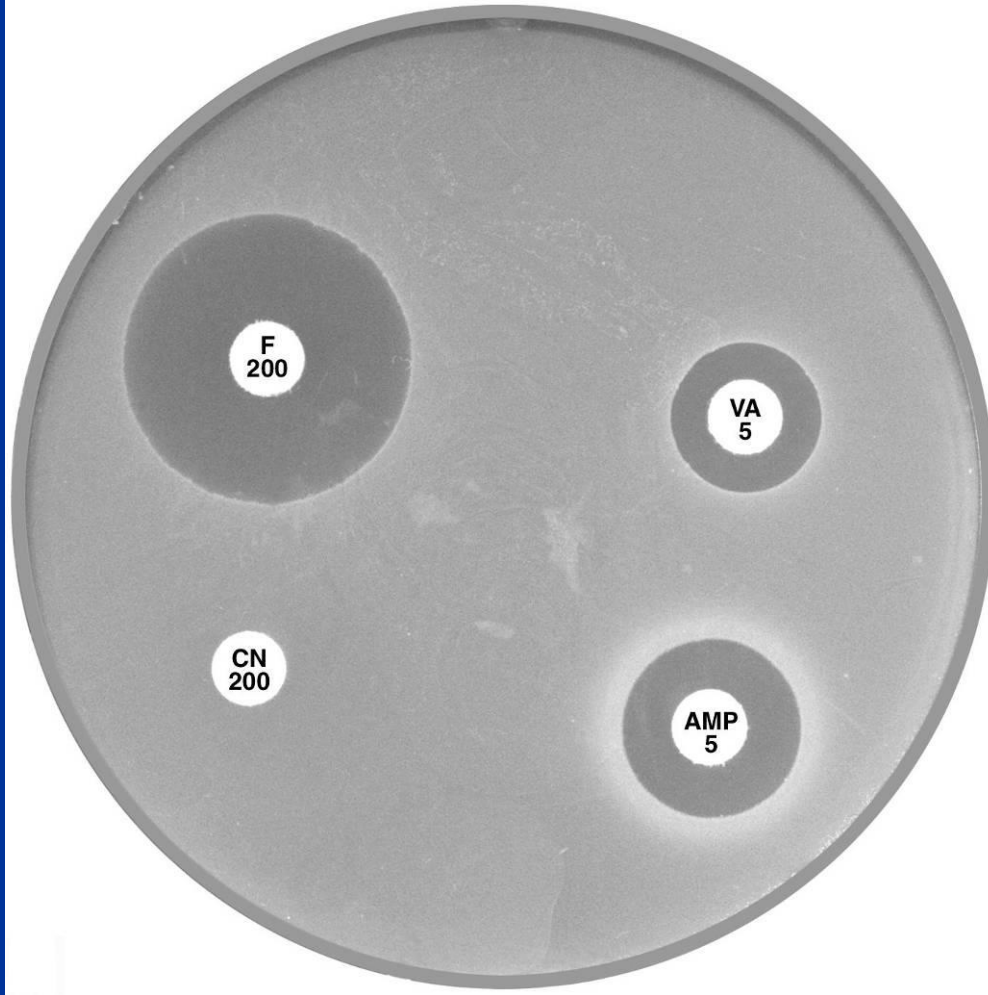
Vancomycin 5 µg



***Enterococcus faecalis* ACM 5184**

The diffuse edge of the zone around ampicillin (AMP 5) indicates susceptibility. Cut off annular radius: **4 mm**

Note the sharp edge around VA 5, susceptible to vancomycin.



A β -lactamase producing *Enterococcus faecalis*

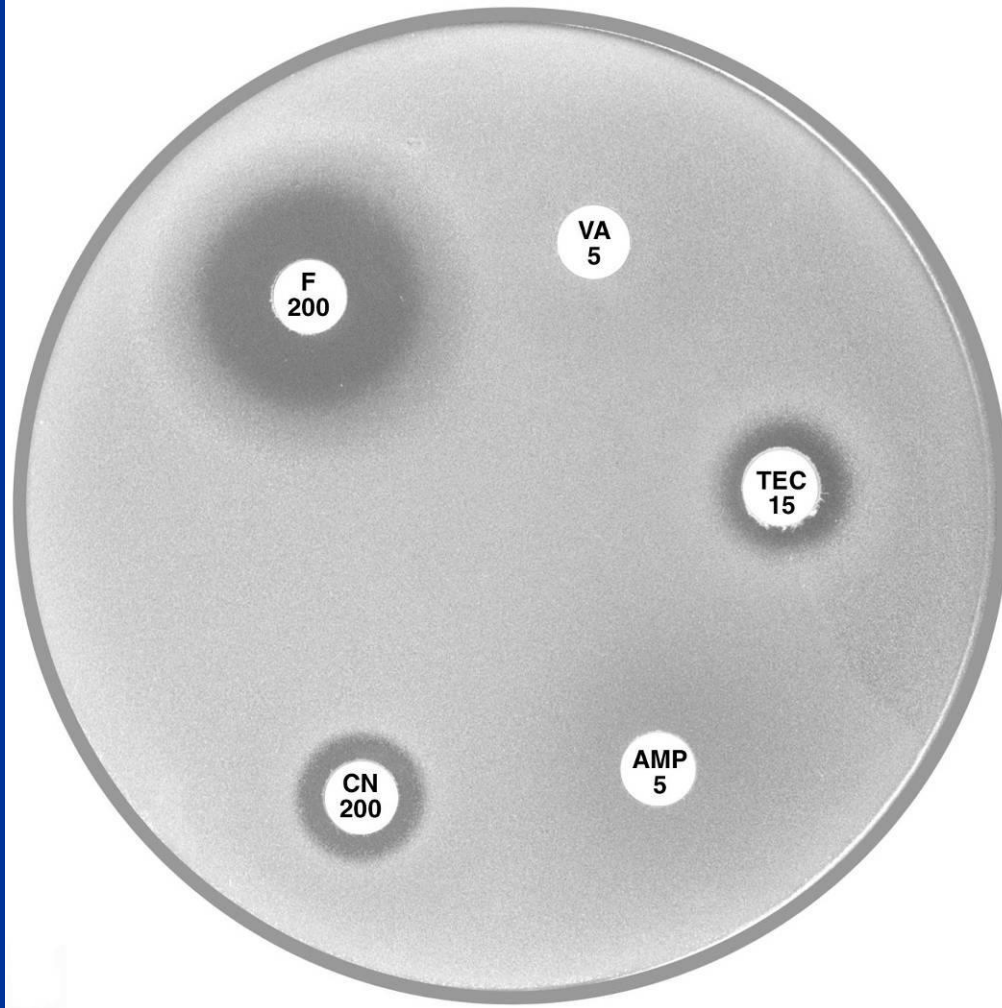
The sharp edge and the reduced zone around ampicillin disc indicates the presence of β -lactamase.



Vancomycin resistant *Enterococcus faecium* of VanB phenotype

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



***Enterococcus faecium* of VanA phenotype**

Resistance to both vancomycin (VA 5) and teicoplanin (TEC15) is typical of vanA phenotype.

R/ VAN, TEC => vanA phenotype

Corynebacterium sp.

Tested on blood Sensitest agar in 5% CO₂

- 14 antibiotics calibrated (Table 1a)
- Penicillin: P 0.5 u and AMP 5

S/ P 0.5

R/ P 0.5

R/ P 0.5

S/ AMP 5 => reduced susceptibility with an MIC between 0.25 and 2 mg/L

R/ AMP 5 => resistant MIC \geq 4 mg/L

* Slow growing isolate: incubate for 48 h

Nocardia sp.

Tested on blood Sensitest agar

- **Inoculum:** saline suspension equivalent to 0.5 McFarland standard for flooding
- **Incubation:** 48 h in air

Amikacin 30 µg

Ciprofloxacin 2.5 µg

Clarithromycin 5 µg

Co-trimoxazole 25 µg

Imipenem 10 µg

Gentamicin 10 µg

Linezolid 10 µg

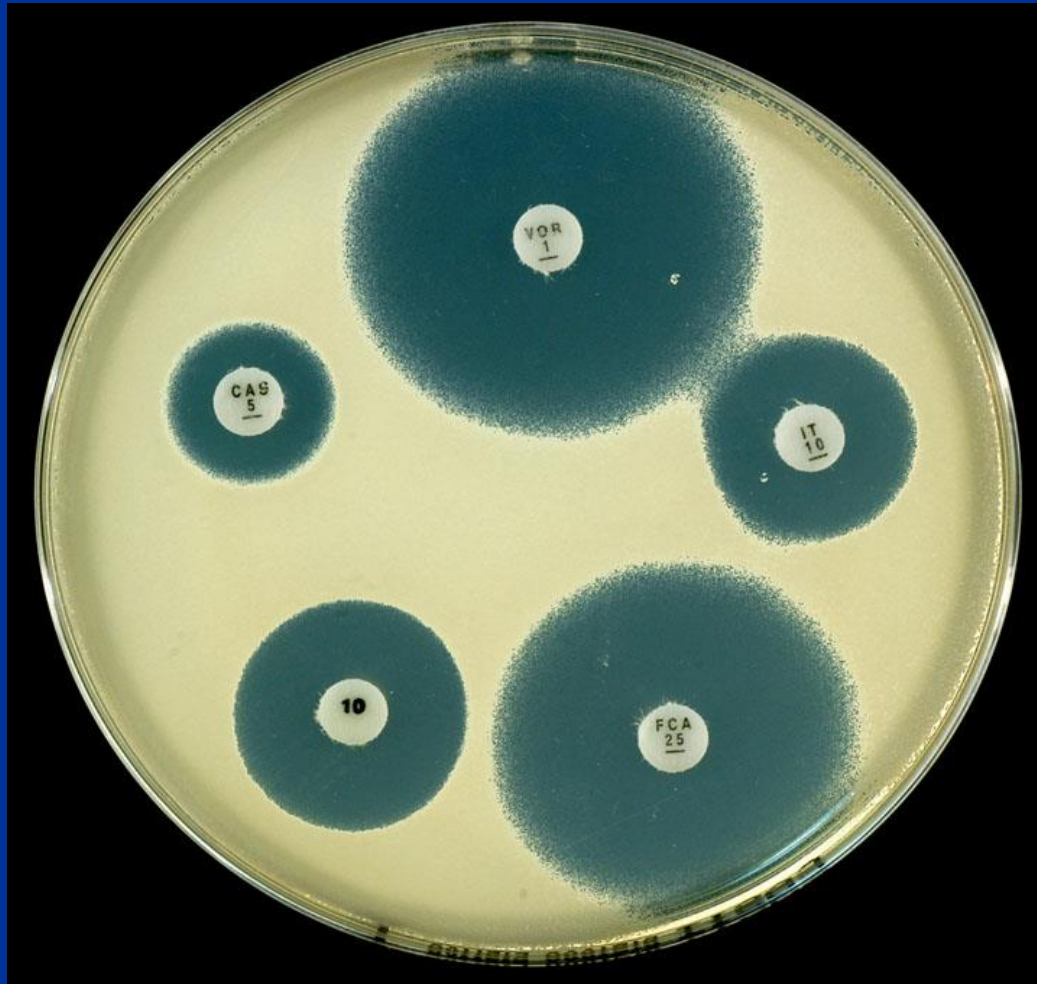
Tigecycline 15 µg

Acknowledgements: We thank Kerry Weeks, George Kotsiou (RNSH) and Matthew Watts for sharing their collection of Nocardia with us.

Update on Antifungal Susceptibility Testing by the CDS method

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Candida parapsilosis (ACM 5283) susceptible to amphotericin B, fluconazole, itraconazole, voriconazole and caspofungin



Cryptococcus neoformans resistant to caspofungin



Media

- Casitone Complex medium.
- Casitone Medium contained Bacto casitone, yeast extract, sodium citrate, di-sodium hydrogen phosphate, potassium di-hydrogen phosphate, glucose and agar.
- This medium is used for testing amphotericin B, fluconazole, itraconazole, voriconazole and caspofungin
- Commercially available (Oxoid)

Inoculum and Incubation

- Grow the yeast strain on SAB for 24 hr at 35°C suspend in saline.
- Adjust the inoculum to 0.5 McFarland standard for *Candida spp* and 1 McFarland for *Cryptococcus spp*.
- OD 0.15 for *Candida* and 0.3 for *Cryptococcus spp*.
- Surface dry the agar media for 1 h.
- Flood with the organism and discard the excess fluid.
- Let plate dry for 10min at RT.
- Apply antifungal discs.
- Incubate plates at 30°C for 24 hr.
- Poor growth may require 48 hr incubation.

Reading of results

- After incubation, measure the annular radius of inhibition of the discs: edge of the disc to edge of confluent growth.
- Ignore any light growth within the inhibitory zone.
- Report either susceptible or resistant according to the calibration table.

Interpretation of Results

Antifungal agent	Disc potency (μg)	Breakpoint interpretation (mm)	MIC for susceptible strains (mg/L)
Amphotericin B	10	4	≤ 0.125
Fluconazole	25	4	≤ 16
Itraconazole	10	2	≤ 2
Voriconazole	1	6	≤ 1
Caspofungin	5	2	≤ 2

Trailing End Points

1. Trailing phenomenon is observed with azoles, particularly fluconazole, itraconazole and voriconazole.
2. There is a light growth inside the zone of inhibition or “double” zones.
3. This phenomenon is reduced by the incubation at 30°C.
4. Rare strains might still show “trailing effect”; report as susceptible to the azoles.

Ref: Rex *et al.* AAC 1998 129-134

The β -lactamases of Gram-negative bacilli

An update

on the detection of common 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**



***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 cephalixin (CL 100), imipenem (IPM 10) and cefotaxime (CTX 5).



The same **IRT** *E. coli*: resistant to ampicillin (AMP 25), Augmentin (AMC 60), Timentin (TIM 85) and Tazocin (TZP 55), susceptible to cephalexin (CL 100), ceftazidime (CAZ 10) and cefotaxime (CTX 5).

ESBLs

(Ambler class A, Bush group 2)

Inhibited by CA

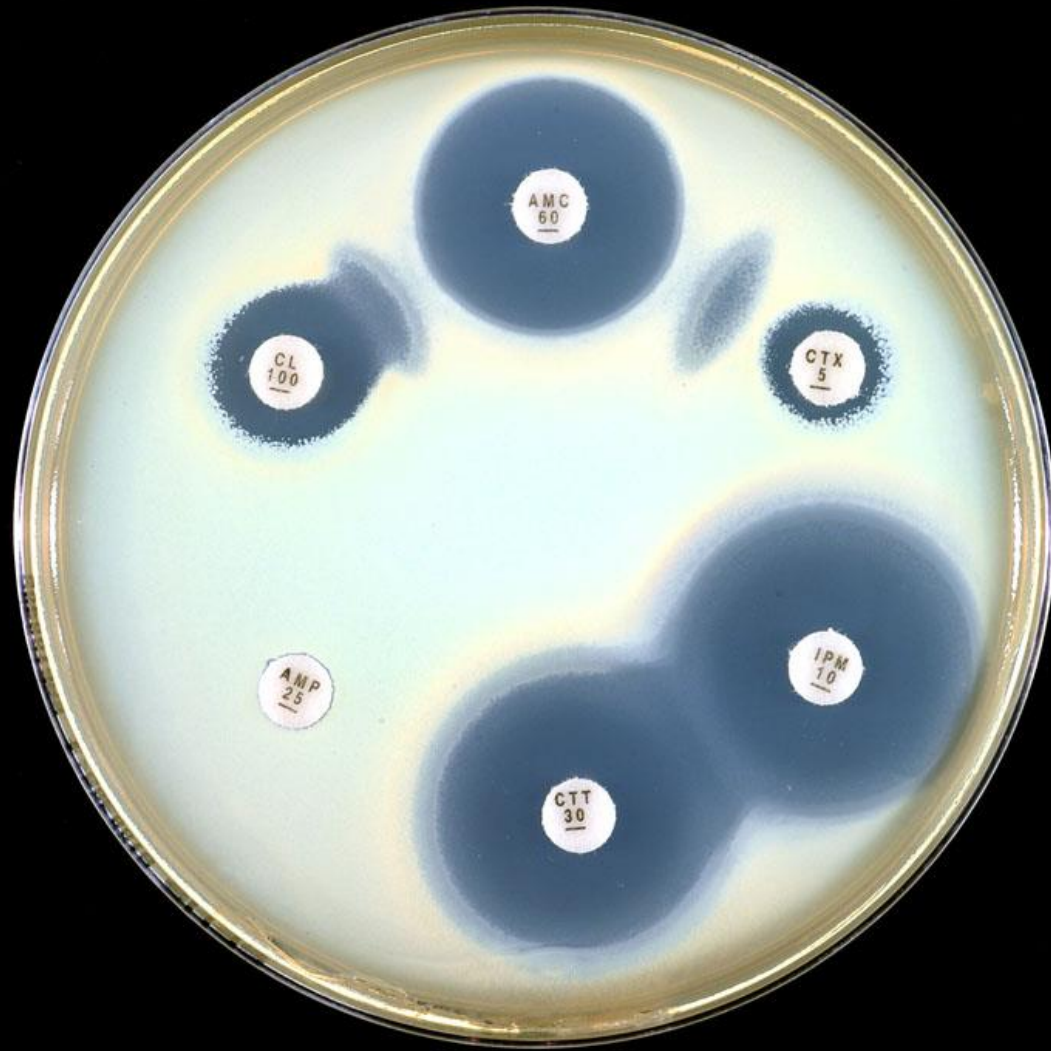
**R/ Cephalosporins (including cefepime) and
aztreonam**

S/ Augmentin (AMC 60)

S/ Cephamycin (cefoxitin, cefotetan)

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

S/ Imipenem (T)



***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).



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) 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 R/ Augmentin (AMC 60), cephalixin (CL 100), cefotaxime (CTX 5); S/ cefepime (FEP 10) and imipenem (IPM 10).



Same *E. coli* with plasmid mediated AmpC 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



**The same *E. coli* with plasmid mediated AmpC
CL 100, AMC 60 and CTX 5 discs placed near 200 ug BA discs**

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*)

Hydrolyses all beta-lactam (except aztreonam)

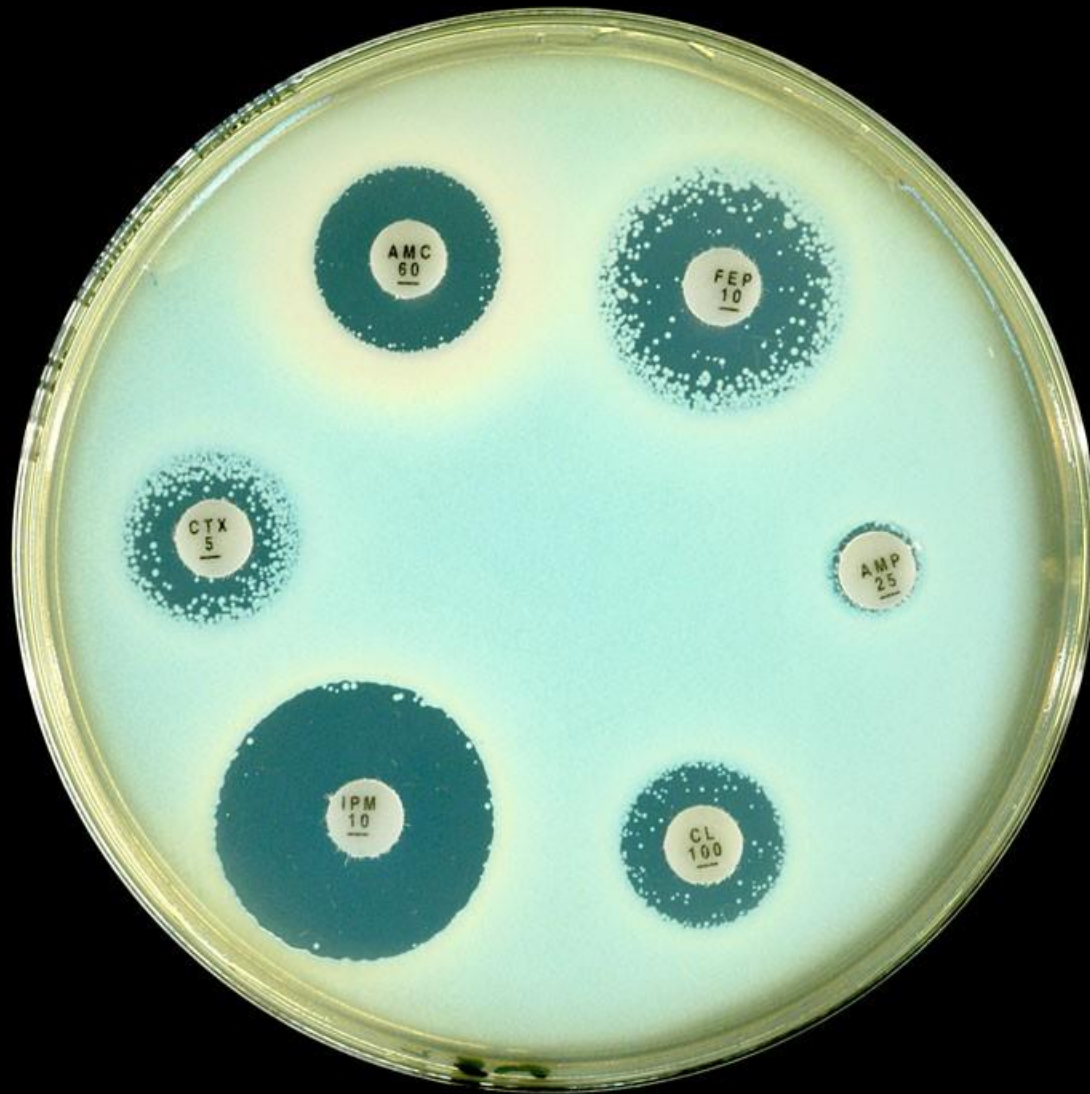
Enterobacteriaceae

May have a zone > 6mm with IPM 10

Pseudomonas aeruginosa

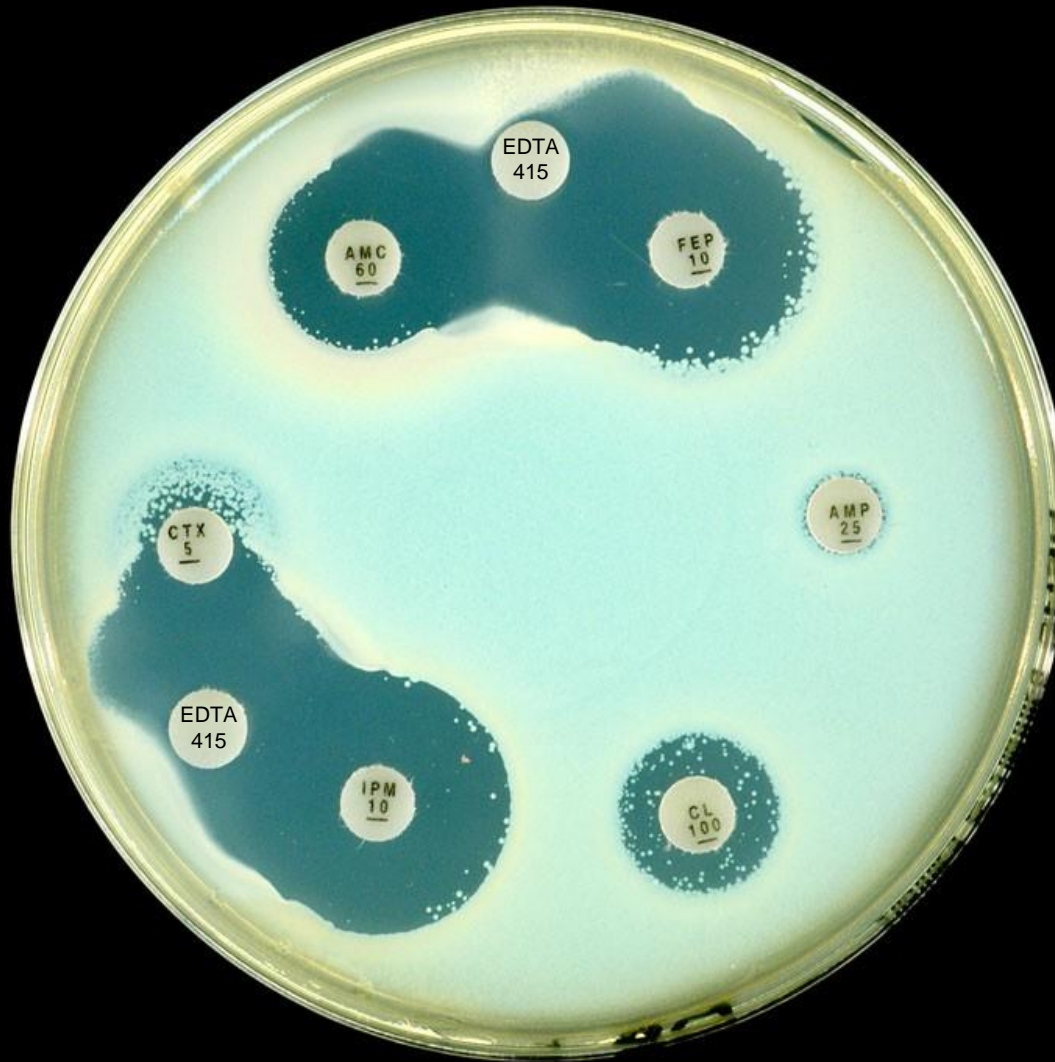
Highly resistant to all β -lactams => no zone

Susceptible only to aztreonam



***C. diversus*: R/AMP 25, AMC 60, CTX 5, CL100 and numerous colonies in cefepime zone (FEP 10) and at the edge of imipenem zone (> 6 mm).**

- **No synergy between FEP/AMC → not ESBL**
- **Numerous resistant colonies in FEP 10 and some at the edge of IPM 10 zone**



Simple phenotypic detection of MBL:

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



MBL producing *E. cloacae*:

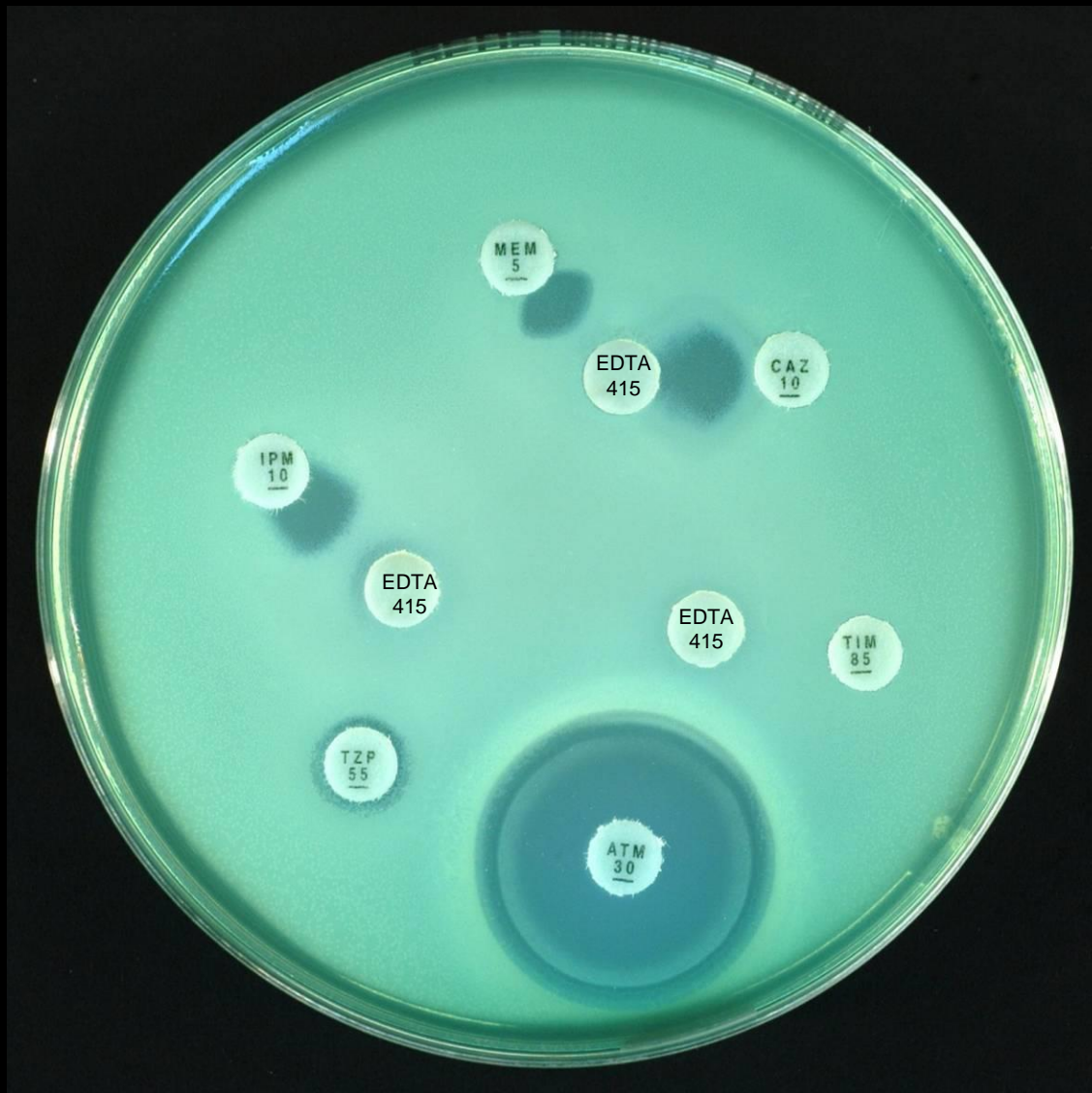
**Synergy between EDTA and cefotaxime (CTX 5)/ ceftazidime (CAZ 10)/
cefepime (FEP 10) / imipenem (IPM 10) S/ aztreonam (ATM 30)**



Pseudomonas aeruginosa candidate for MBL detection:

No zone around imipenem (IPM 10) ceftazidime (CAZ 10), tazocin (TIZP 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 => sensitive to clavulanate

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



The same *K. pneumoniae*: KPC-2 producing *K. pneumoniae* (France)*

The top half: control discs IPM 10, FEP 10, CTX 5.

The bottom half: same discs with 10 µg sodium clavulanate added .

***Ref: AAC 2005, 4423-24**

KPC in *Klebsiella pneumoniae*

Plasmid mediated *K. pneumoniae* carbapenemase (KPC)

Ambler class A or Bush group 2f

- Reported in Europe, US (Brooklyn 24%)
- Not yet reported in Australia
- 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

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

=> Test ertapenem or send for confirmation

Two organisms with unusual mechanisms of resistance



***E. coli* from UTI: resistant to cephalexin (CL 100), susceptible to ampicillin (AMP 25), Augmentin (AMC 60)...**

Investigation: β -lactamase negative (nitrocefin) => R/ CL 100 (? efflux)

Report: R/ cephalexin S/ampicillin



S. epidermidis from a patient with an infected shunt on long term rifampicin/fusidate treatment tested on Sensitest agar 35°C (24h)
The organism grows only around rifampicin (RD 1) => “rifampicin dependent”
Needs to be tested on Sensitest agar supplemented with blood.



The same *S. epidermidis* tested on blood Sensitest agar showing resistance to ciprofloxacin (CIP 2.5), gentamicin (CN 10) rifampicin (RD 1) and susceptibility to tetracycline (TE 10), fusidate (FD 2.5), vancomycin (VA 5).