Welcome

to

the CDS Workshop

Brisbane 2012

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ITS GREAT TO BE BACK IN QUEENSLAND

What is the CDS Test ?

High potency disc diffusion test as are CLSI and Eucast

but

Generally it does not attempt to grade antibiotic susceptibilities into more than 2 grades.

Uses a simplified method of interpretation of zone sizes obviates the need for interpretative tables.

Disc potencies are selected to ensure optimal delineation between susceptible and resistant isolates.

Augments interpretation of results by assessment of inhibitory zone morphology.

Background of the CDS Organisation

The organisation is clinically orientated and is funded by the public hospital system.

It is independent and receives no support from, nor is beholden to, any pharmaceutical or diagnostic supply company.

The method has been in development and in use for over 40 years.

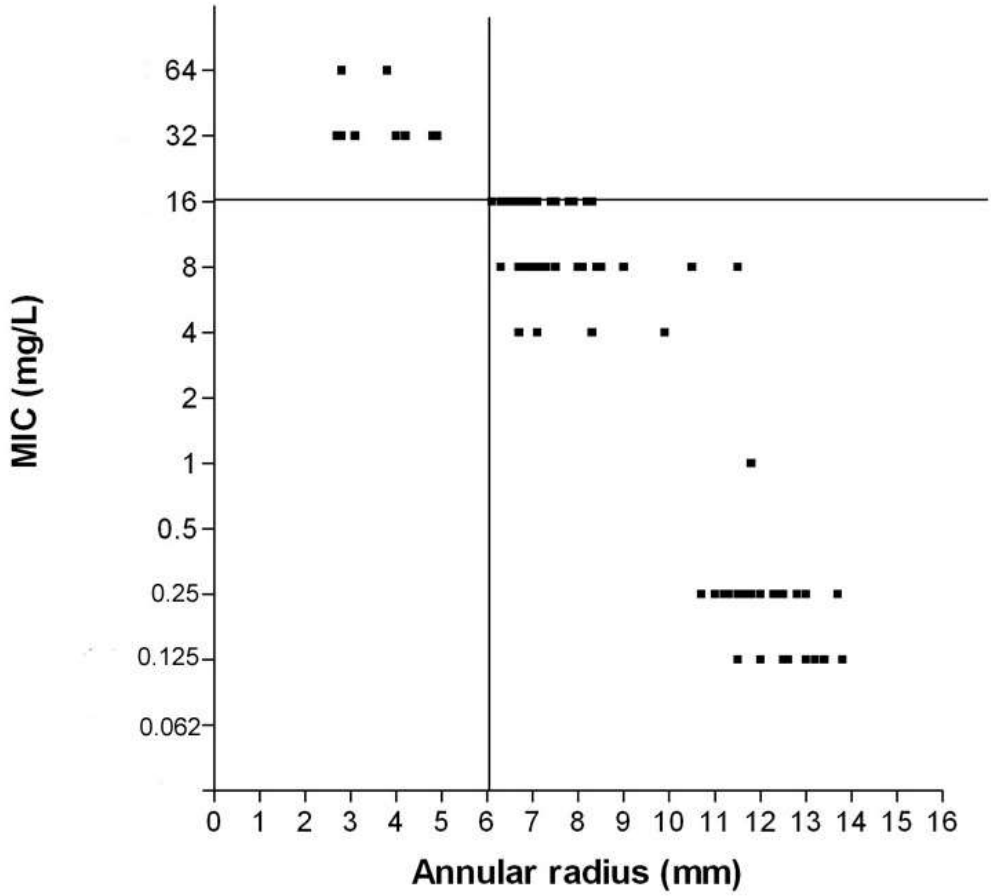
All services provided are free.

It has links to both medical and veterinary laboratories.

Value Added Services of the CDS Organisation

- The CDS Users Group is a national cooperative and the method has been developed in response to feedback from its members**.**
- The method is kept up to date with calibration of antibiotics at or prior to their introduction into clinical practice.
- The method is under constant review responding to reports (often by CDS members) of emerging mechanisms of resistance.
- The CDS reference laboratory at Randwick responds to all enquiries from CDS members including requests to confirm unusual susceptibility test results.
- The reference laboratory maintains and distributes free of charge all organisms used in quality assurance testing of the CDS method.
- A readily accessible website devoted to antibiotic susceptibility testing is maintained on the University of NSW server.
- A hard copy of the CDS Manual is published periodically and is supplemented with newsletters and a website edition of updated versions of the Manual.
- A CDS workshop has been conducted at the ASM Scientific Meeting for the past 30 years.

Calibration of Doxycycline 60 µg with Enterococci



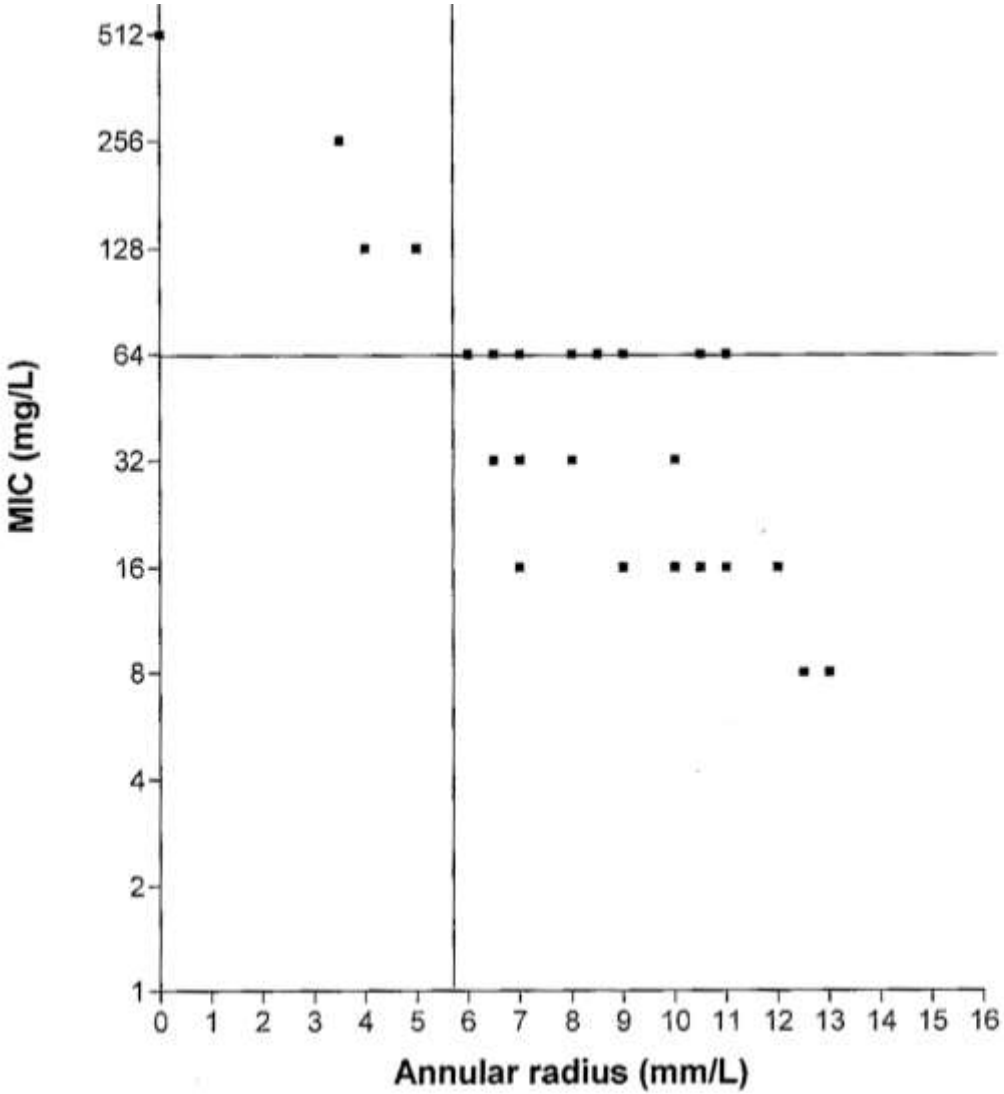
Susceptibility of Enterococci to doxycycline (n=105, 49 *E. faecalis*, 56 *E. faecium*)

Species	Number Susceptible	Number Susceptible
	Low Level (MIC ≤ 1mg/L)	High Level (MIC ≤ 16mg/L)
E. faecalis	11 (10%)	45 (92%)
E. faecium	25 (24%)	50 (89%)

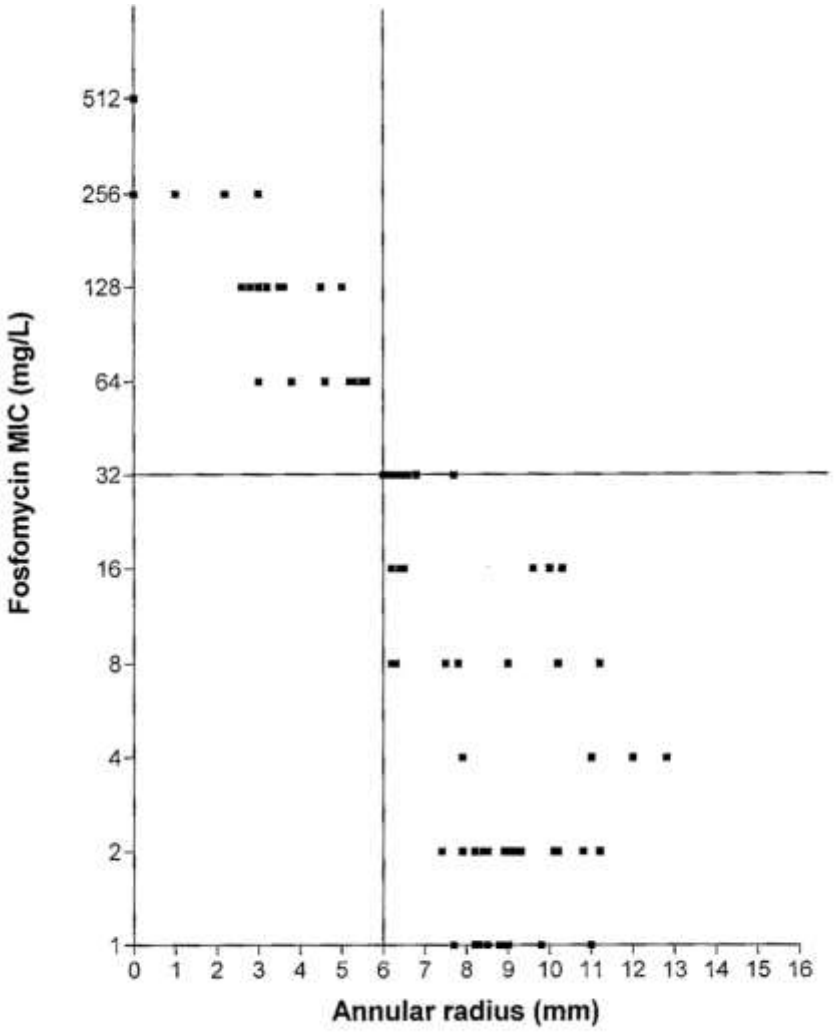
Doxycycline 60μg & Enterococcus Susceptible

Doxycycline 60μg & Enterococcus Resistant

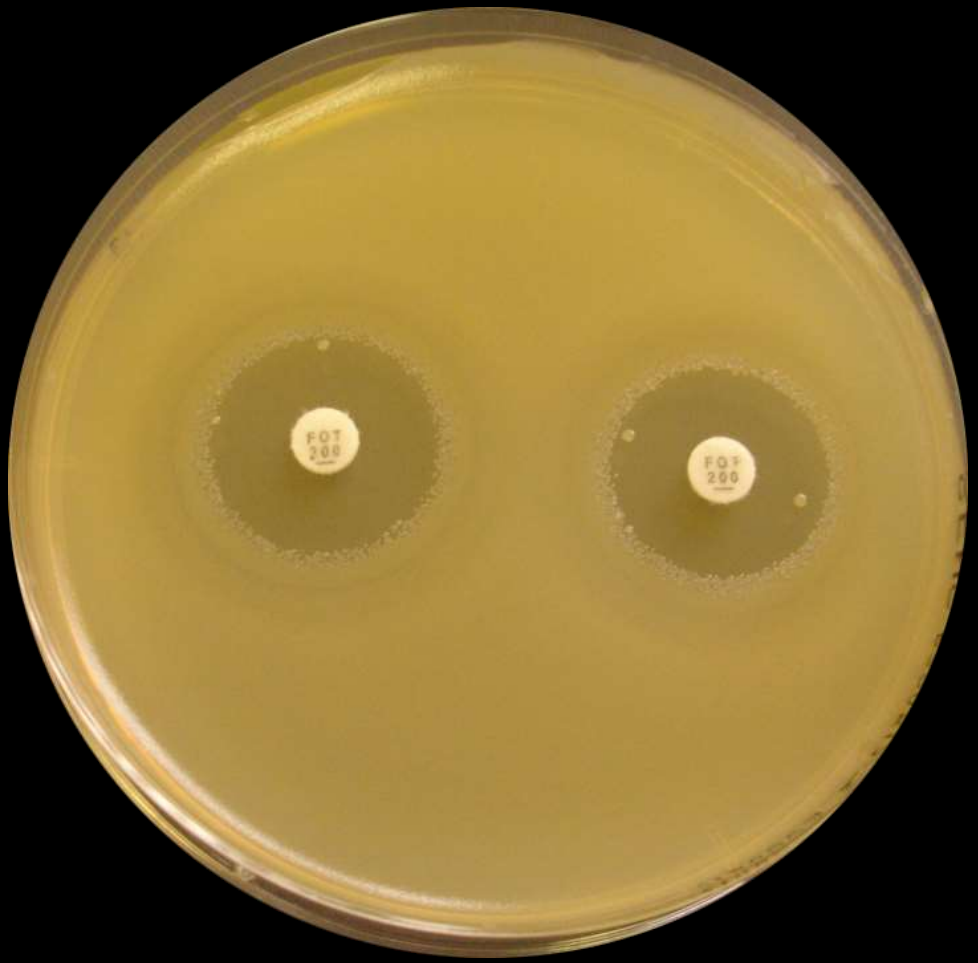
Fosfomycin & Enterococci



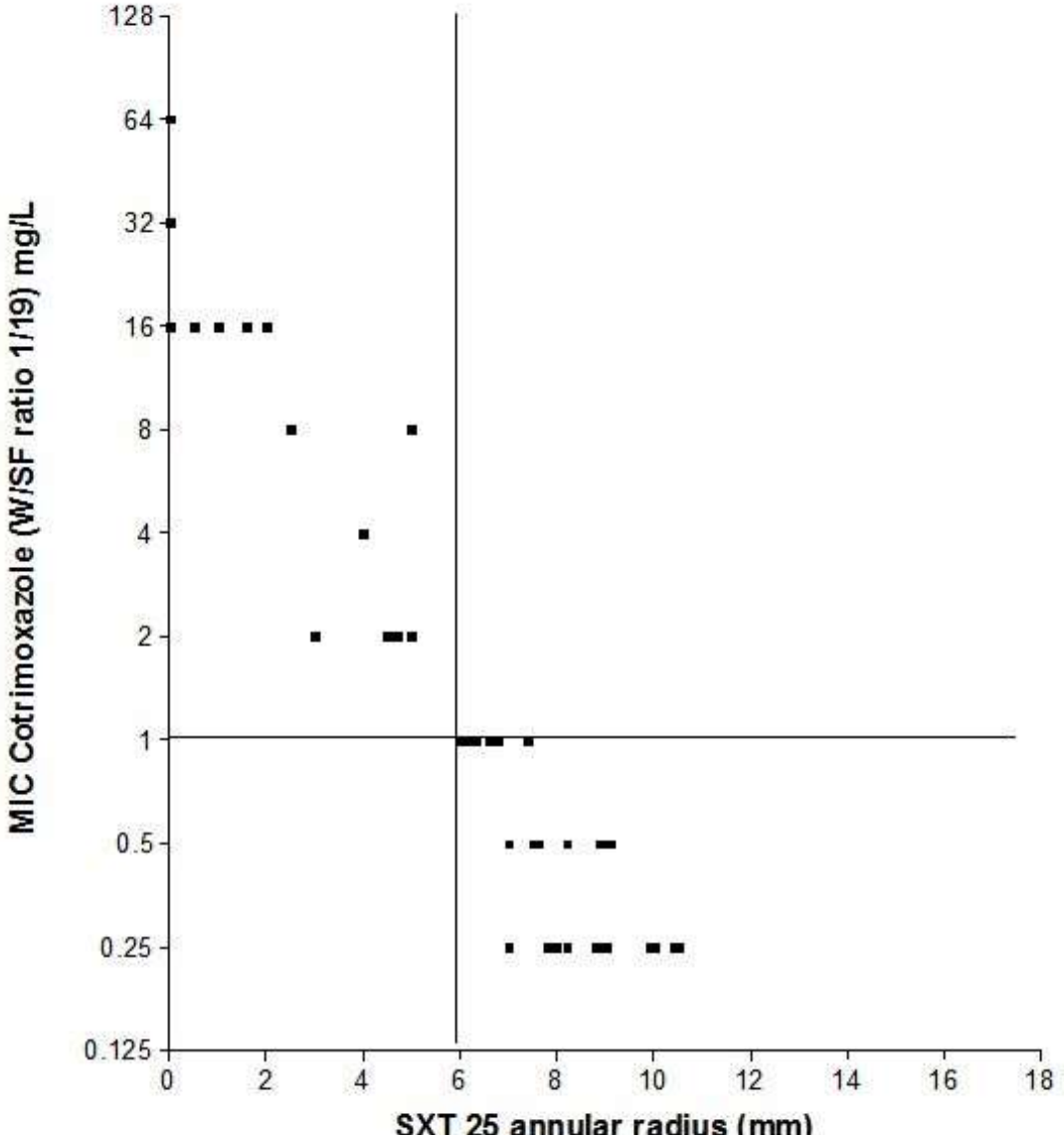
Fosfomycin & Enterobacteriaceae



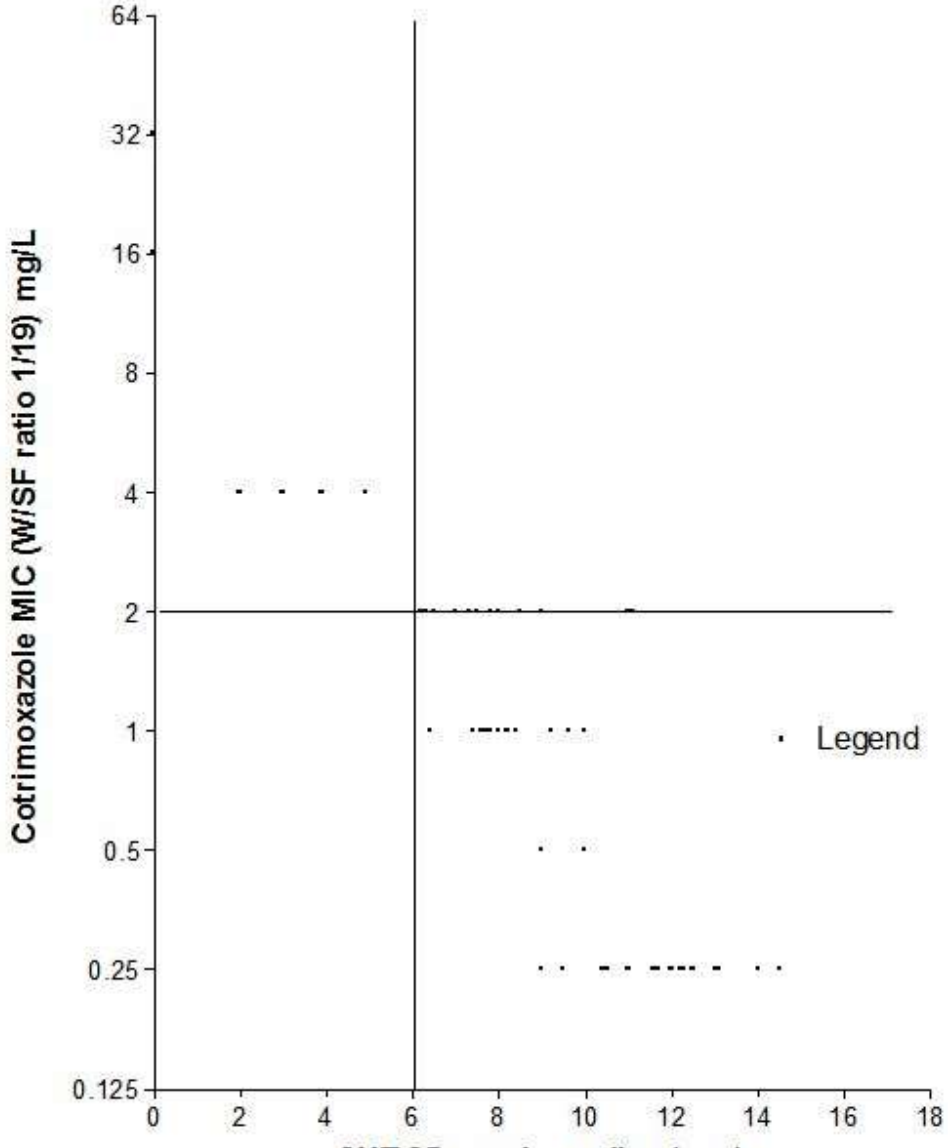
Fosfomycin & E. coli ACM 5185



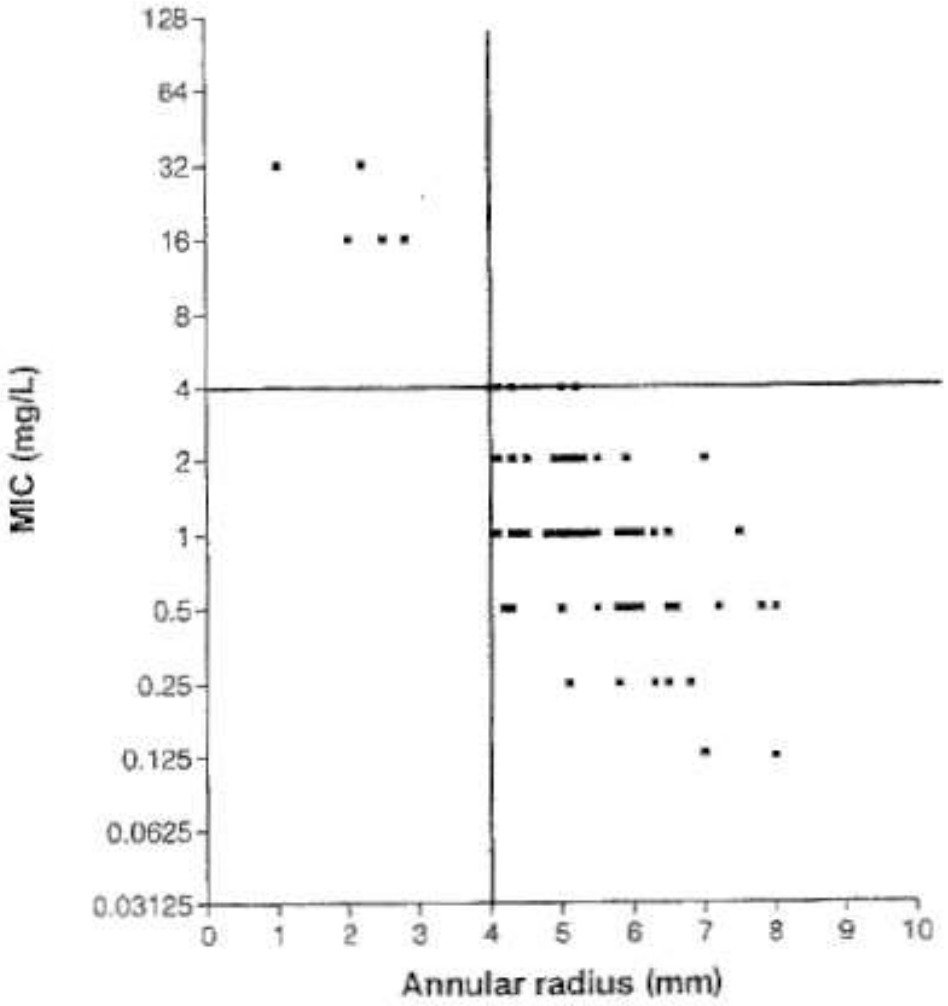
Cotrimoxazole & Enterobacteriaceae



Cotrimoxazole & Pseudomonas-like Spp



Daptomycin 30 µg + $CaCl_2$ & Gram +ve



Disc Testing Daptomycin Susceptible Staph.aureus

Disc Testing Daptomycin Susceptible E.faecalis

Disc Testing Daptomycin Resistant E.faecalis

An update of the CDS test

CDS Workshop

ASM 2012 Brisbane

S. aureus:

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)

Staphylococci Vs cefoxitin 10 & oxacillin 1

Standard 6 mm cut off

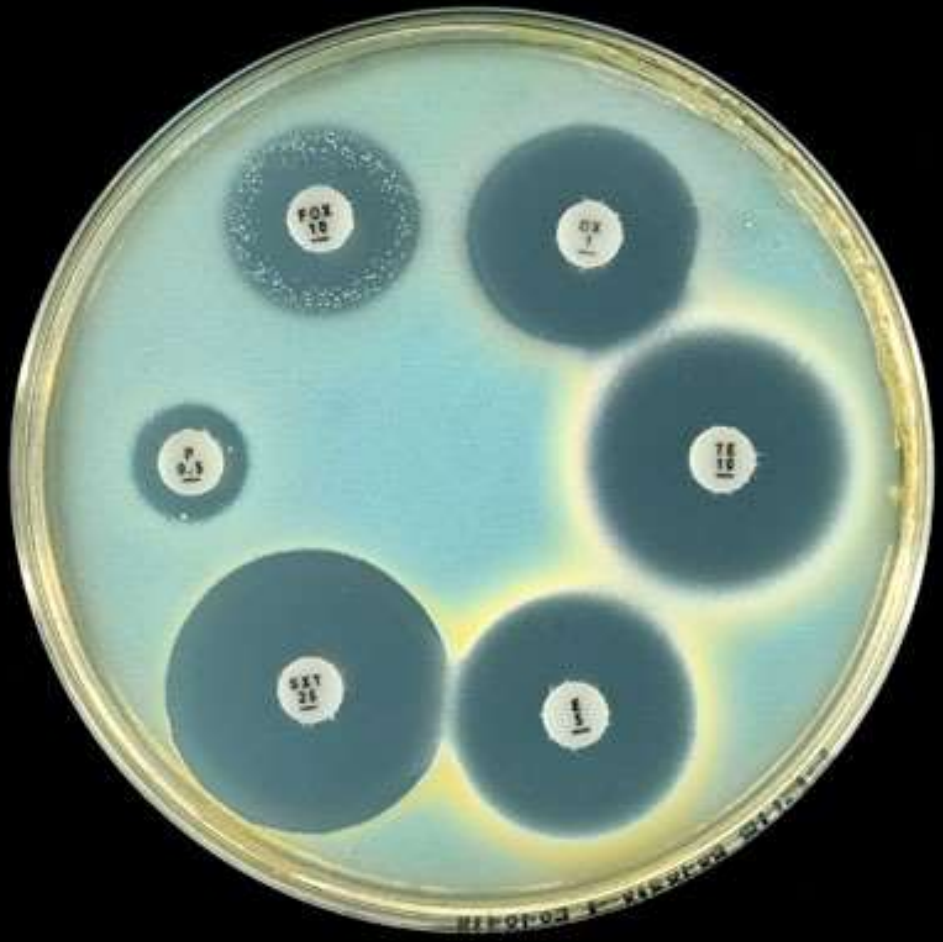
Cefoxitin 10 (Fox 10) for *S. aureus*

* 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



Oxacillin-susceptible *mec-A* positive *S. aureus* (MRSA):

Non multi-resistant MRSA with a heterogeneous resistance

- numerous resistant colonies in FOX 10 zone, large OX 1 zone
- cefoxitin is a better inducer of PBP 2a than oxacillin

RCPA-QAP 2012:2:1A
S. saprophyticus

Susceptibility Testing

• Twenty six participants reported penicillin resistance with 6 (3) being CDS users.

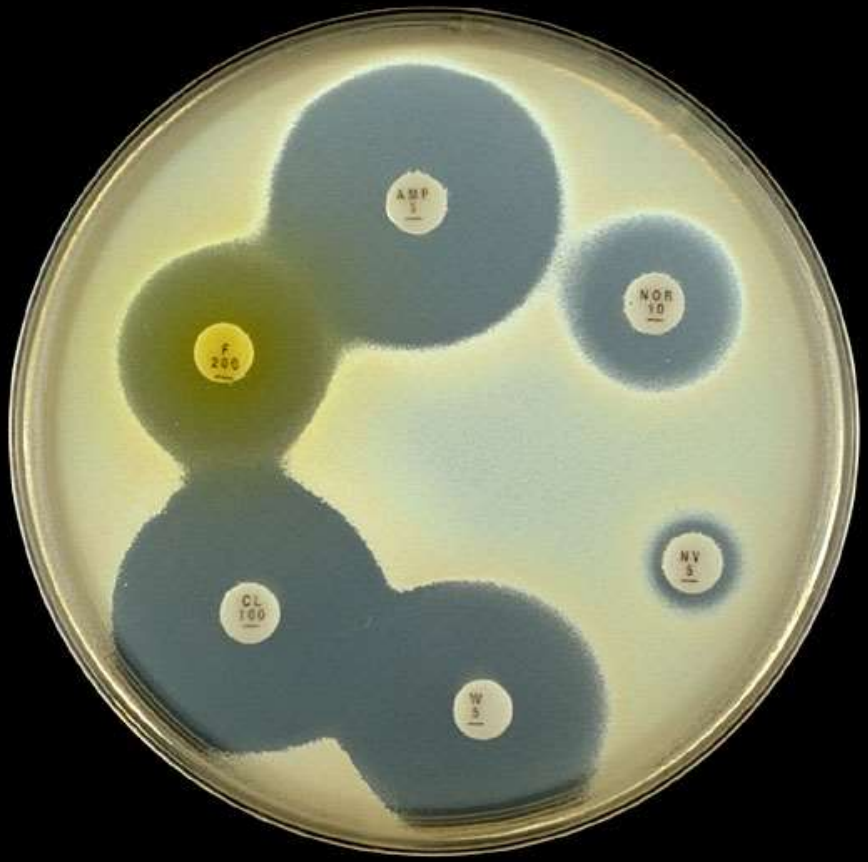
• CLSI guidelines do not recommend the routine testing of urine isolates.

Staphylococcus saprophyticus

Novobiocin resistant CNS isolated from urine

Wild strains have higher penicillin and oxacillin MIC than other CNS:

- Ampicillin 5 (AMP 5 instead of P 0.5)
  Report: amoxycillin, penicillin V
- Cephalexin 100 (CL 100 instead of Ox 1)
  Report: Augmentin



S. saprophyticus (common type, R/ NV 5)

- S/ ampicillin (AMP 5) and cephalexin (CL 100)
- S/ nitrofurantoin (F 200), trimethoprim (W 5), norfloxacin (NOR 10)

S. saprophyticus (β-lactamase positive)

- R/ ampicillin (AMP 5) S/ cephalexin (CL 100)
- S/ nitrofurantoin (F 200), trimethoprim (W 5), norfloxacin (NOR 10)

S. saprophyticus (*mecA* gene-positive)

- R/ ampicillin (AMP 5) and cephalexin (CL 100)
- S/ nitrofurantoin (F 200), trimethoprim (W 5), norfloxacin (NOR 10)

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* (K1): standard interpretation
6. PM AmpC: standard interpretation

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 clavulanic acid
  S/ AMC 60

- AmpC: (Bush group 1, Ambler class C)
  Not inhibited by clavulanic acid , inhibited by boronic acid
  R/ AMC 60 S/ FEP 10

- MBL: (Bush group 3, Ambler class B)
  Not inhibited by clavulanic acid , inhibited by EDTA
  R/ AMC 60 R/ FEP 10

- Oxa (Not true ESBL): (Bush group 4, Ambler class D)
  Partially inhibited by clavulanic acid
  S/ AMC 60 Reduced FEP 10 zone (synergy)
  S/ CL 100

ESBLs *sensu-stricto*

(Ambler class A, Bush group 2)

Inhibited by clavulanic acid

R to Cephalosporins (including cefepime) and aztreonam

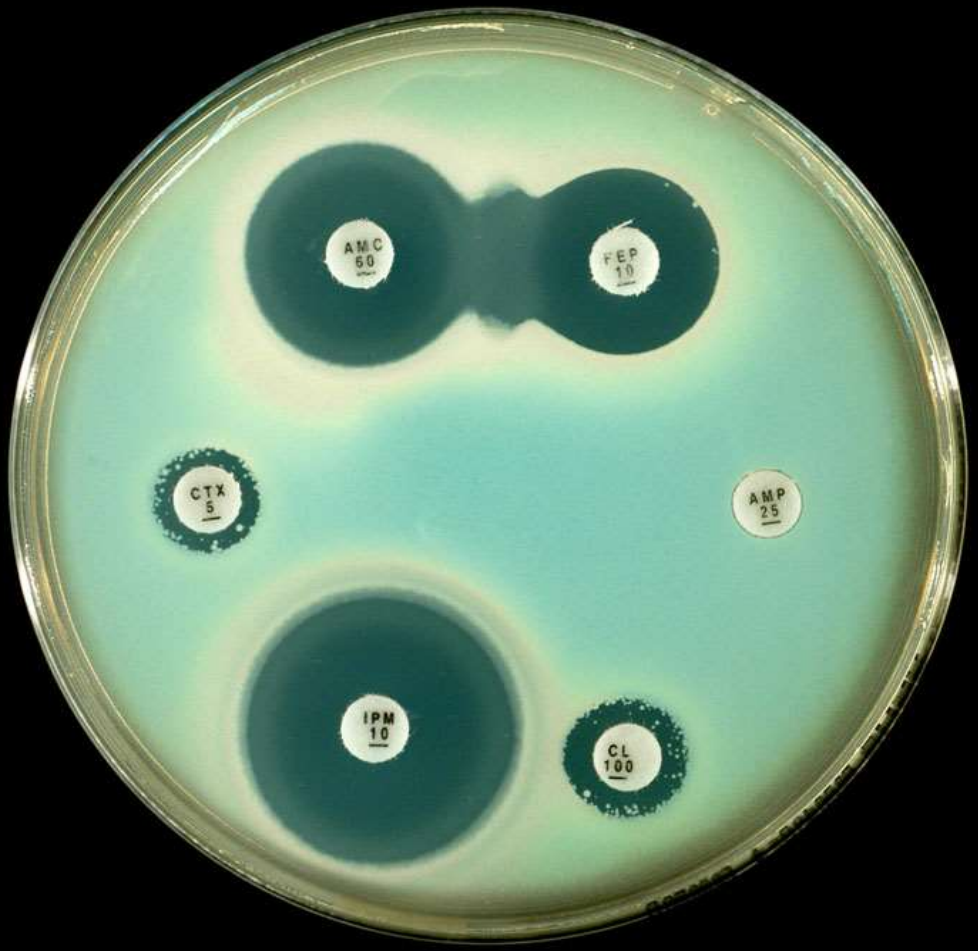
S to Augmentin (AMC 60)

s to Cephamycin (cefoxitin, cefotetan)

CDS routine testing → Marked synergy with AMC 60

(confirmation not needed)

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 plasmid mediated AmpC in *E. coli*

R to AMC 60 (not inhibited by clav acid)

R to CL 100

R to CTX 5 (high level resistance)

R to cefamycin (CMY-1, DHA, MOX…)

S/ FEP 10 (4^{th} generation cephalosorin)

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= 200 µg boronic acid disc

Acquired Metallo-Beta-Lactamases (MBLs)

Ambler class B or Bush group 3

Inhibited by EDTA (zinc binding)

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)

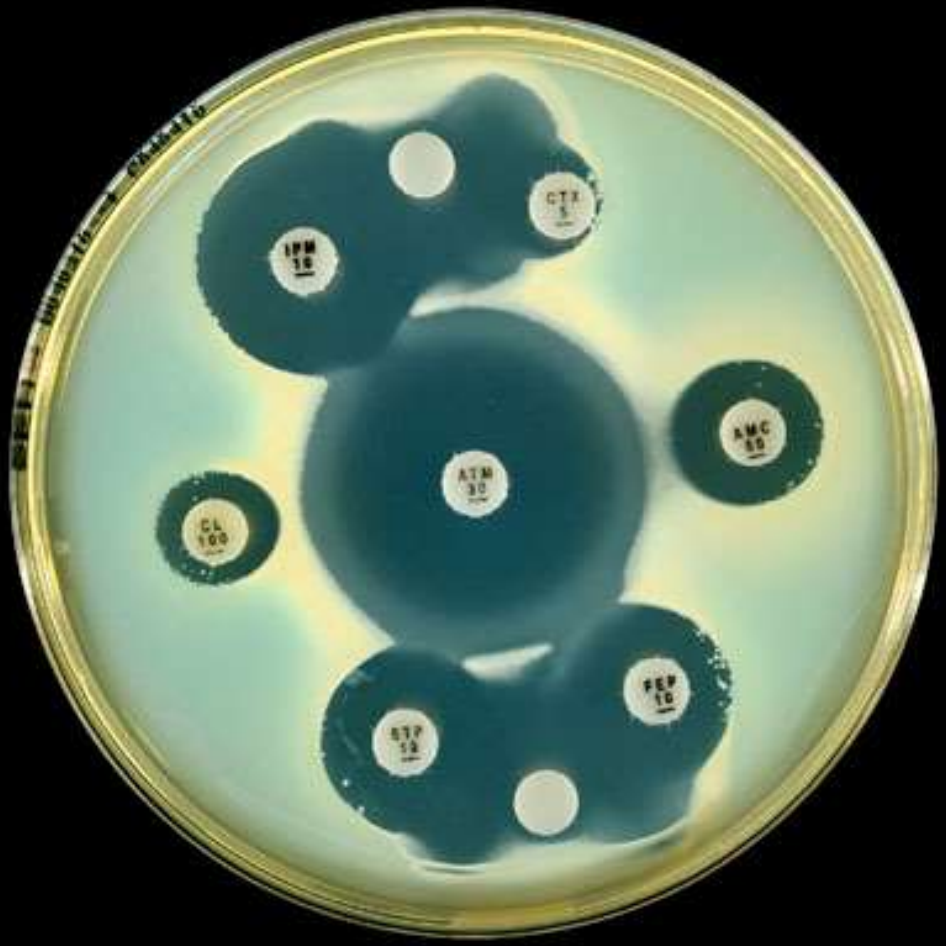
Highly resistant to all β-lactams i.e. no zone

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

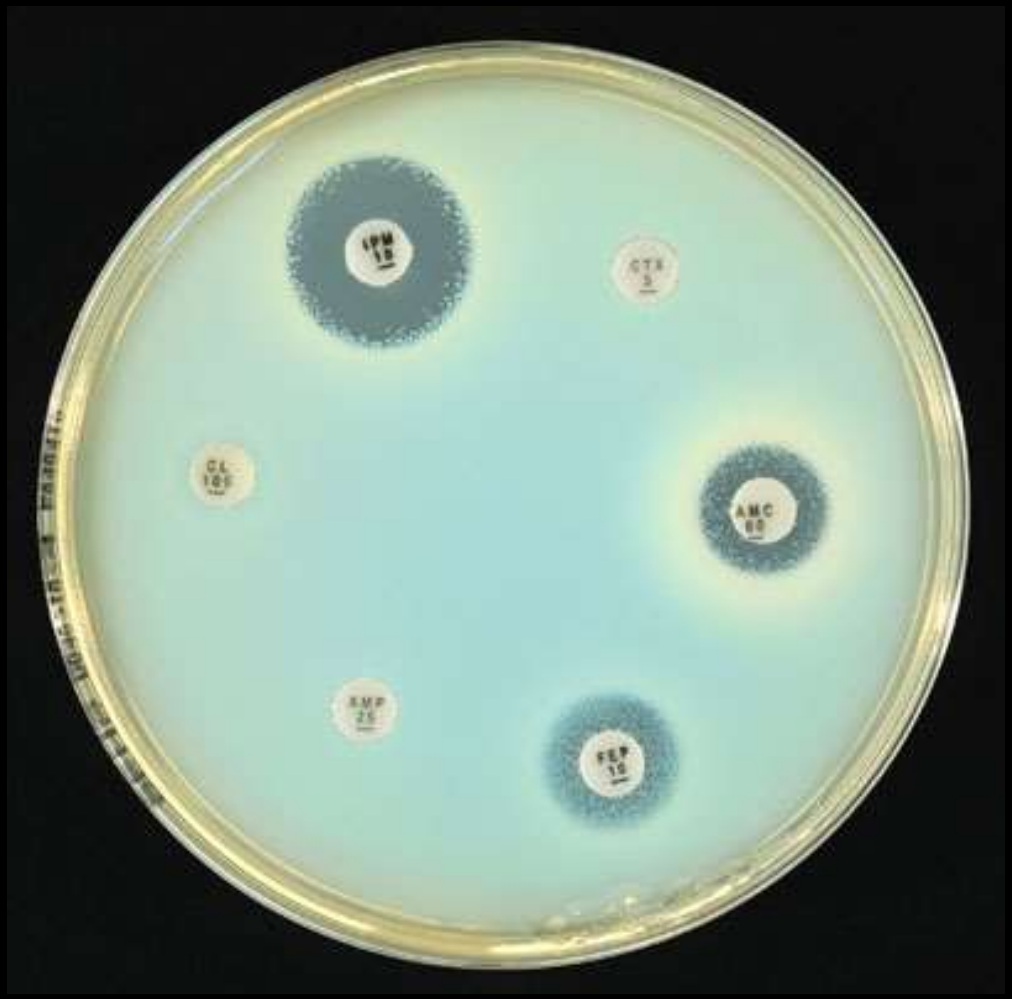
Resistant colonies at the edge of IPM 10 zone: ? MBL



Confirmation:

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

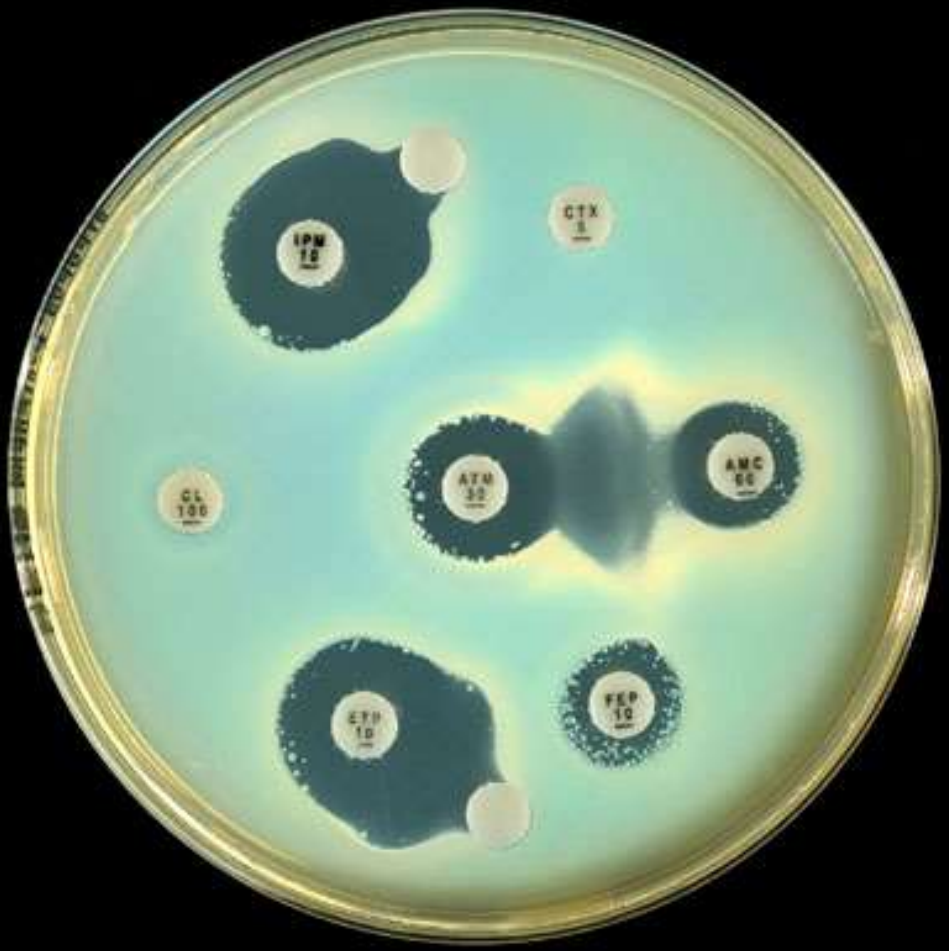
=> Metallo-β-lactamase



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



Confirmation:

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

=> MBL and ESBL

KPC

Plasmid mediated *K. pneumoniae* carbapenemase (KPC)

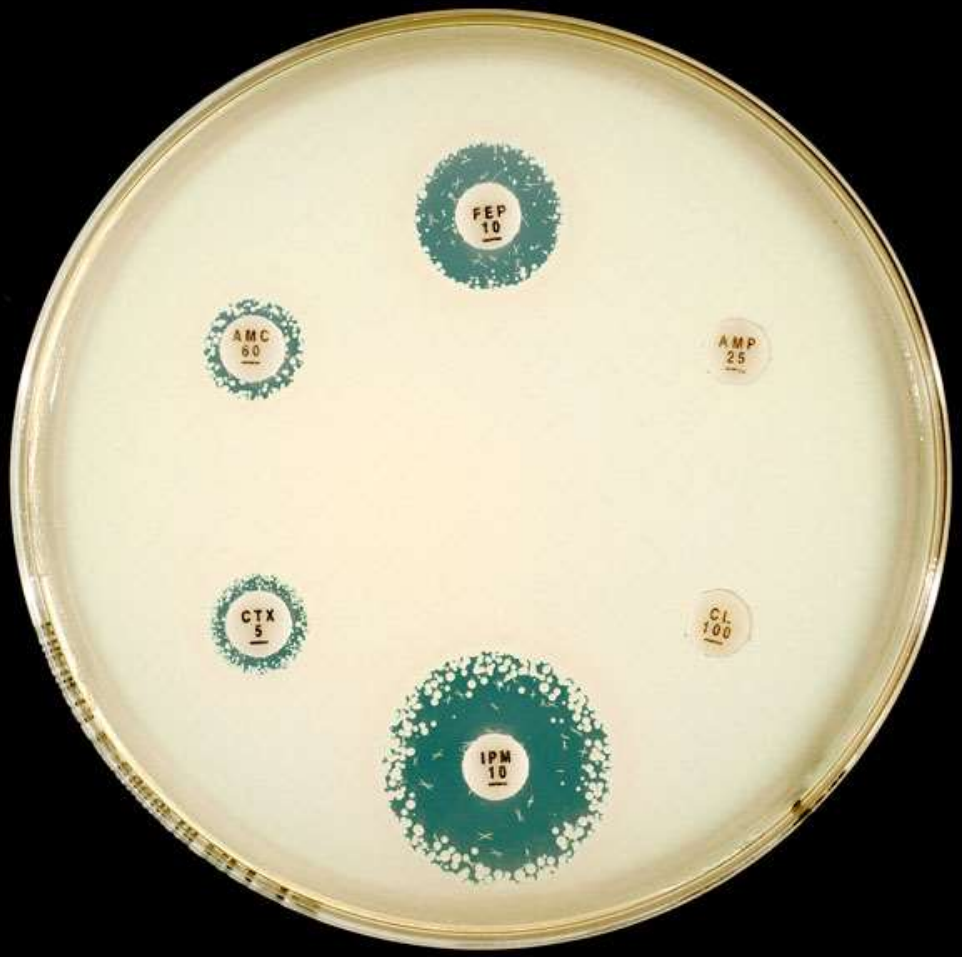
Ambler class A or Bush group

- Reported in Europe, US (KP, *E. coli* in Israel AAC 2006)
- Australia (first isolate in Sept 2010)
- Inhibited by clavulanic acid i.e ESBL affecting carbapenems
  KPC-1 , KPC-2, KPC-3
  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 and R/IPM or colonies at edge of IPM zone
In doubt, test ertapenem and/or send for confirmation

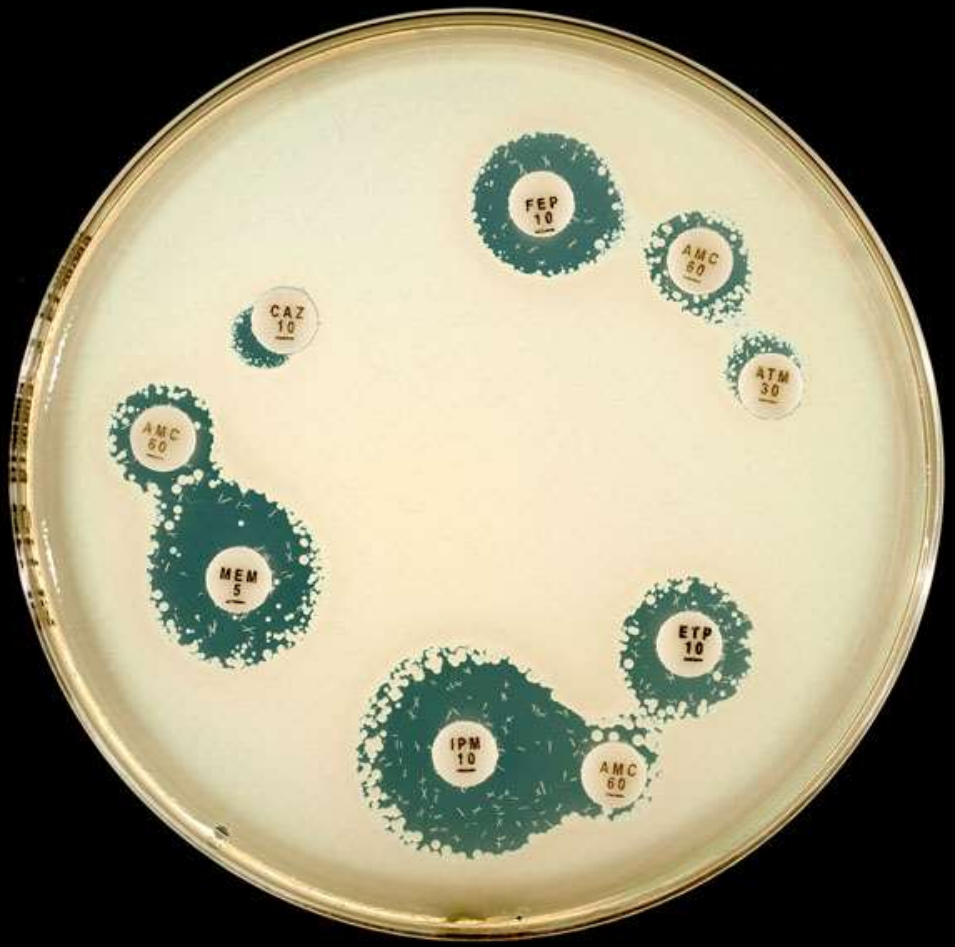
Confirmation: No synergy with EDTA, R/ ertapenem
Mild synergy with AMC 60

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

i.e. 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 discs

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 showing synergy with EDTA

R/ aztreonam (ATM 30)

? MBL

Synergy between an EDTA disc placed next to aztreonam (ATM 30), tazocin (TPZ 55), ceftazidime (CAZ 10), Timentin (TIM 85) discs.

No synergy with imipenem (IPM 10).

Synergy between EDTA and aztreonam (ATM 30)

Pseudomonas aeruginosa showing non-specific synergy with EDTA

E. coli:

Resistant to ampicillin (AMP 25) and Augmentin (AMC 60)

Susceptible to cephalexin (CL 100), imipenem (IPM 10) and cefotaxime (CTX 5).

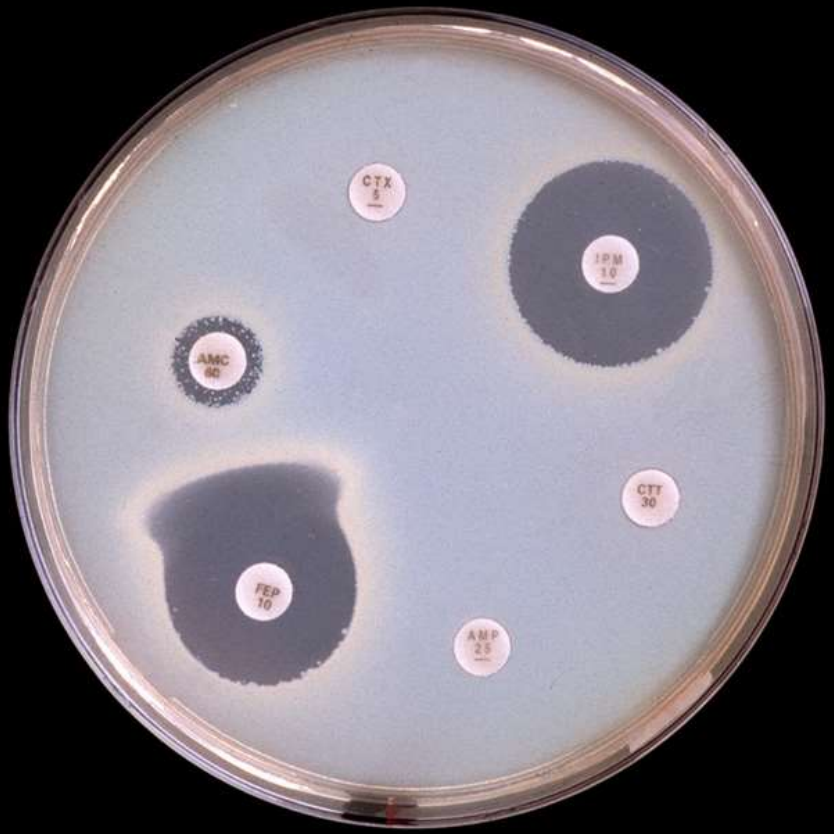
What is the mechanism of resistance?

Answer:

R/ ampicillin (AMP 25) and Augmentin (AMC 60), Timentin (TIM), Tazocin (TZP)

S/ ALL cephalosporins:cephalexin (CL 100), cefotaxime (CTX 5), ceftazidime (CAZ 10)

IRT (Inhibitor Resistant TEM)

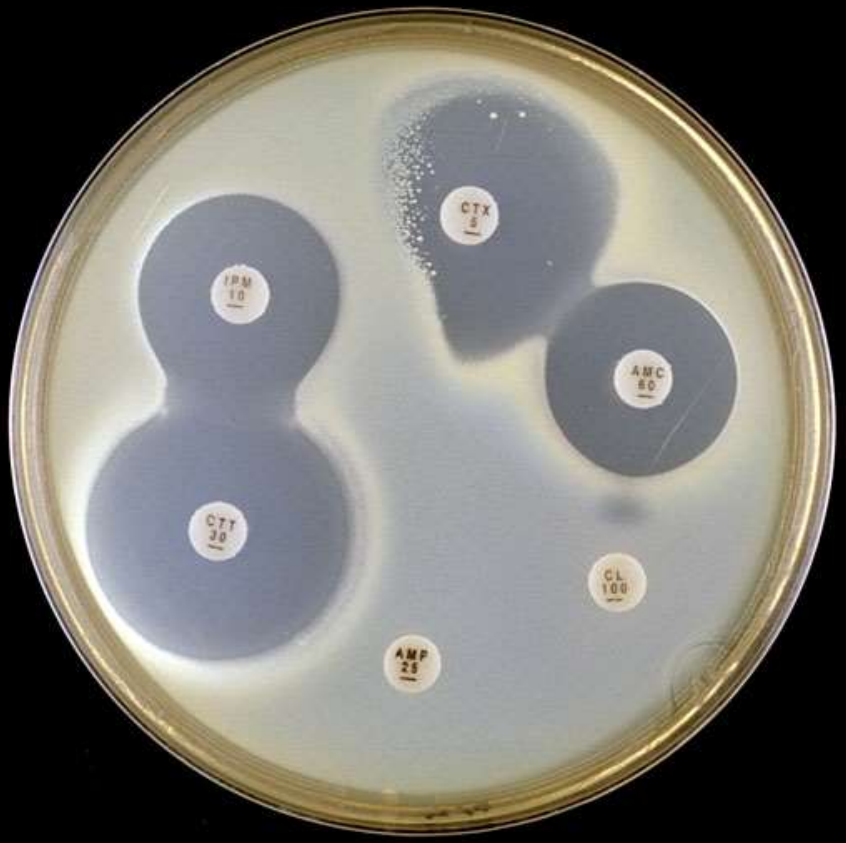


Enterobacter cloacae

Hyper-producer of AmpC (Bush, functional group 1) β-lactamase , ESBL present

R/ AMP 25, CTT 30, CTX 5, AMC 60

Synergy between AMC 60 and FEP 10 (not with CTX 5)



Proteus penneri

Inducible class A (Bush group 2) β-lactamase, inhibited by clavulanate

R/ AMP 25, CL 100

S/ AMC 60, CTT 30, IPM 10

Report R/ CTX, AMP, CL

Staphylococcus saprophyticus

Novobiocin resistant CNS isolated from urine

Wild strains have higher penicillin and oxacillin MIC than other CNS:

- Ampicillin 5 (AMP 5 instead of P 0.5)

  Report: amoxycillin, penicillin V

- Cephalexin 100 (CL 100 instead of Ox 1)

  Report: Augmentin