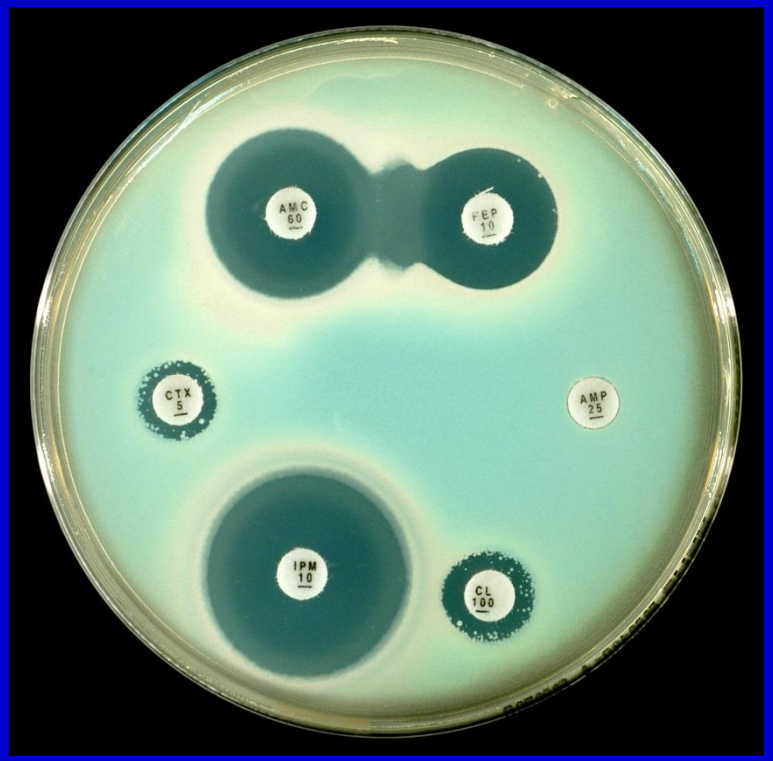
Welcome to the CDS Workshop

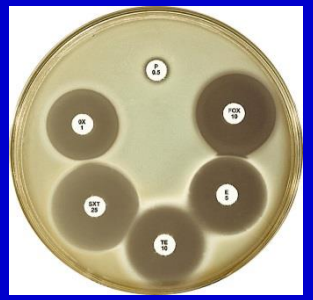


ASM Adelaide 2019

NSW GOVERNMENT Health Pathology

CDS Workshop Topics

- Ceftolozane-tazobactam calibration
- Polymyxin testing – What to do?
- Carbapenems
- Updates CDS Manual

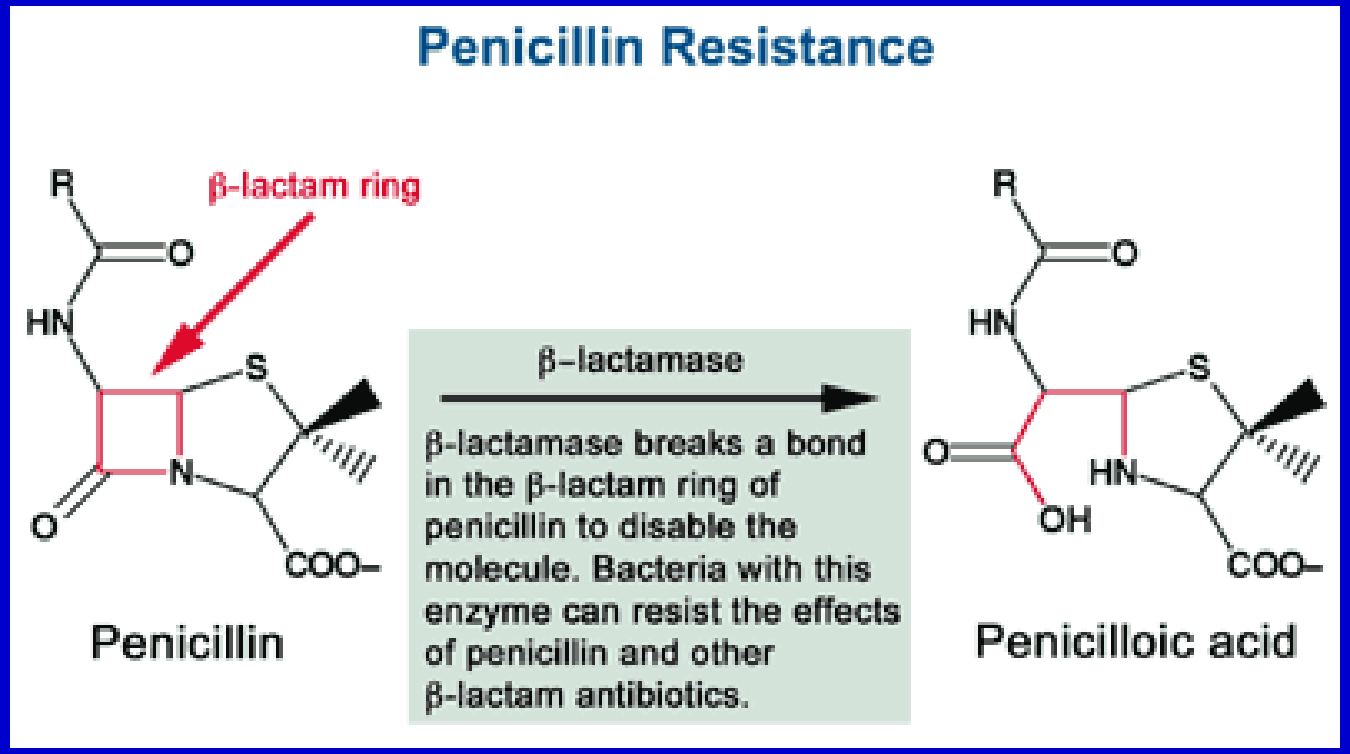


Calibration Ceftolozane/tazobactam

ASM Adelaide 2019

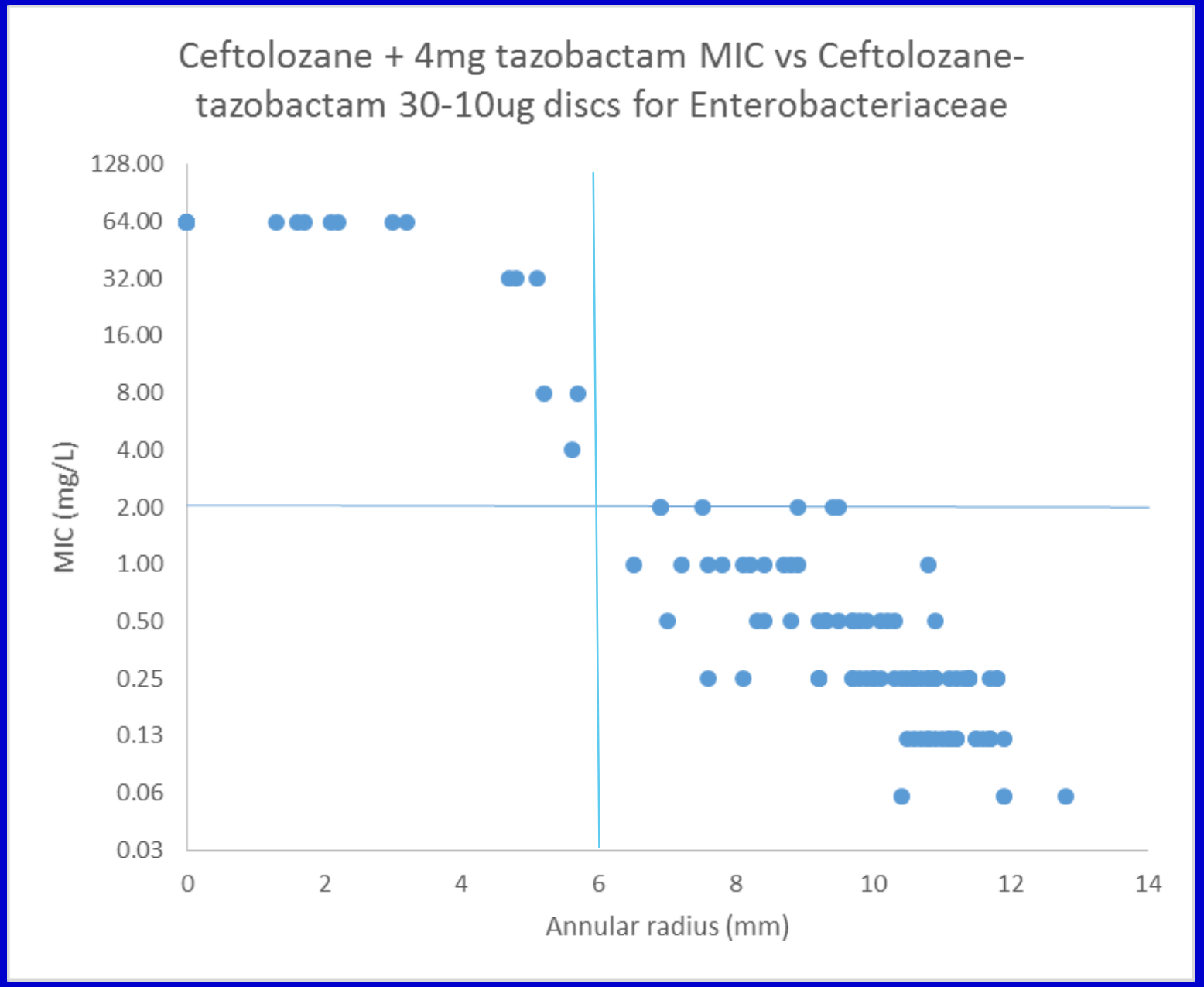
Dianne Rafferty

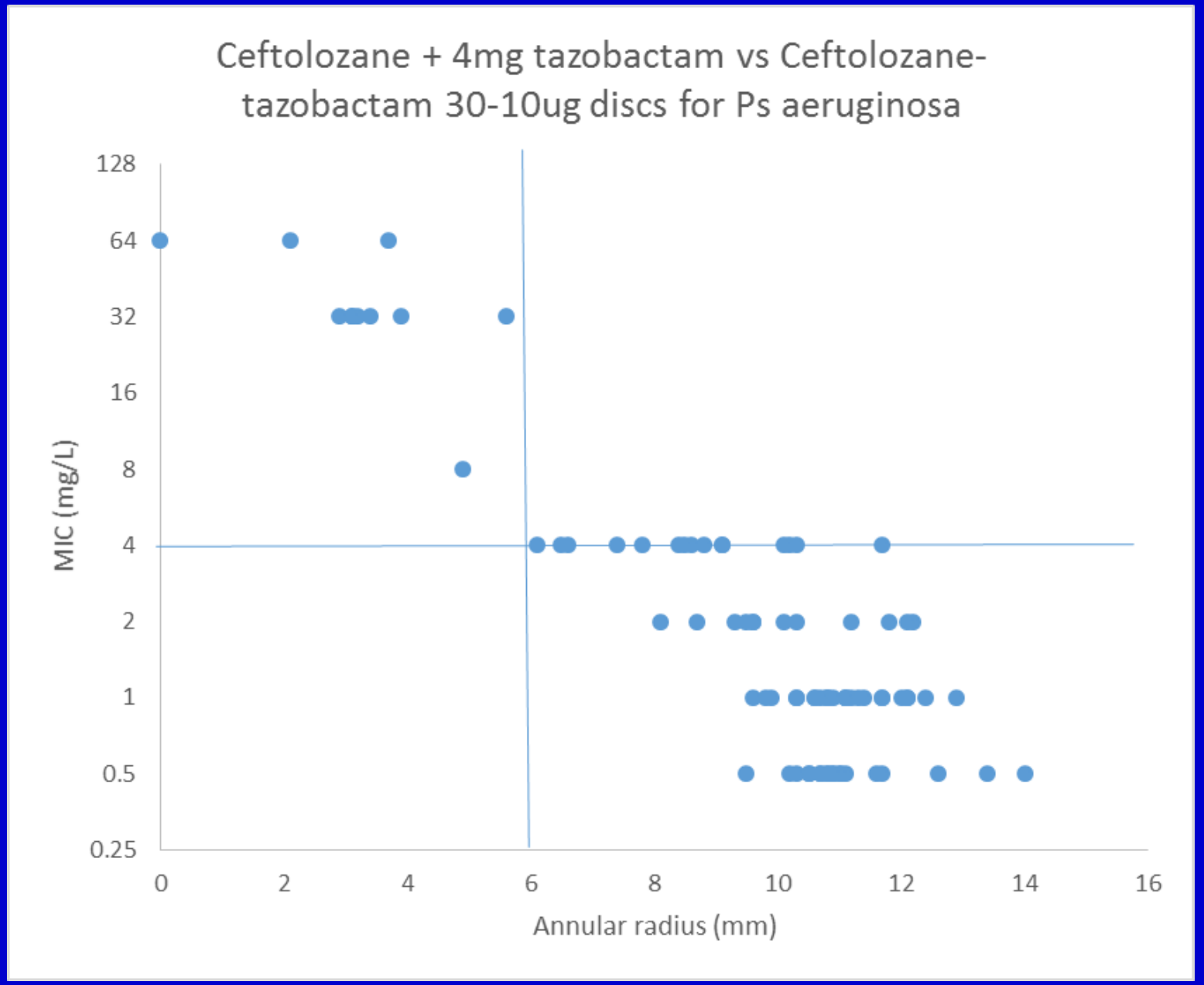
B-lactamase production



Ceftolozane-tazobactam

- Cephalosporin/ß-lactamase inhibitor combination
- Active against Enterobacteriaceae & resistant Ps aeruginosa
- Active against select ESBLs
  - Class A (TEM, SHV, CTX-M), some class C (AmpC) and class D (OXA).
  - No activity against MBLs (NDM, IMP,VIM) or carbapenemases (KPC).


Ceftolozane + 4mg tazobactam MIC vs Ceftolozane-tazobactam 30-10ug discs for Enterobacteriaceae
MIC (mg/L)
128.00
64.00
32.00
16.00
8.00
4.00
2.00
1.00
0.50
0.25
0.13
0.06
0.03
0
2
4
6
8
10
12
14
Annular radius (mm)


Ceftolozane + 4mg tazobactam vs Ceftolozane-tazobactam 30-10ug discs for Ps aeruginosa
MIC (mg/L)
128
64
32
16
8
4
2
1
0.5
0.25
0
2
4
6
8
10
12
14
16
Annular radius (mm)

Organism	Number	%S	%R
E coli	49	91	9
Enterobacter spp	21	76	24
Klebsiella spp	36	65	35
Other GNB	8	75	25
Ps aeruginosa	82	87	13

%S, Susceptible, %R, Resistant. Based on a breakpoint of 2+4mg/L for Enterobacteriaceae and 4+4mg/L for P aeruginosa

Ceftolozane-tazobactam

- Enterobacteriaceae
  - MIC Susceptible strains ≤ 2.0 mg/L
  - Annular radius of susceptible strains ≥ 6mm
- P aeruginosa
  - MIC Susceptible strains ≤ 4.0 mg/L
  - Annular radius of susceptible strains ≥ 6mm

References

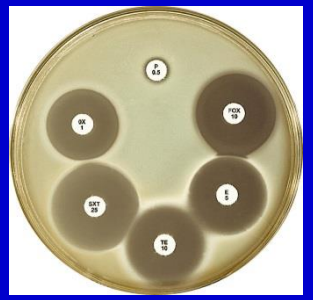
1. Bell S.M., Pham J.N., Rafferty D.L., Allerton J.K., *Antibiotic Susceptibility Testing by the CDS Method – A Manual for Medical and Veterinary Laboratories Ninth Edition, 2018*
2. Lisco J.L, et al, *Ceftolozane/tazobactam and ceftazidime/avibactam: two novel β-lactam/β-lactamase inhibitor combination agents for the treatment resistant Gram-negative bacterial infections,* Int Journal of Antimicrobial Agents 46 (2015) 266-271
3. Livermore D.M, et al, *Activity of ceftolozane/tazobactam against surveillance and 'problem' Enterobacteriaceae, Pseudomonas aeruginosa and non-fermenters from the British Isles,* J Antimicrob Chemother 72(8) (2017) 2278-2289

Acknowledgements

Sincere thanks to

- Syd Bell, Dianne Rafferty and Pratibha James for their support and collaboration
- Management and staff in the Microbiology Laboratory at NSWHP

  St George Hospital
- CDS Users group



Polymyxin Review

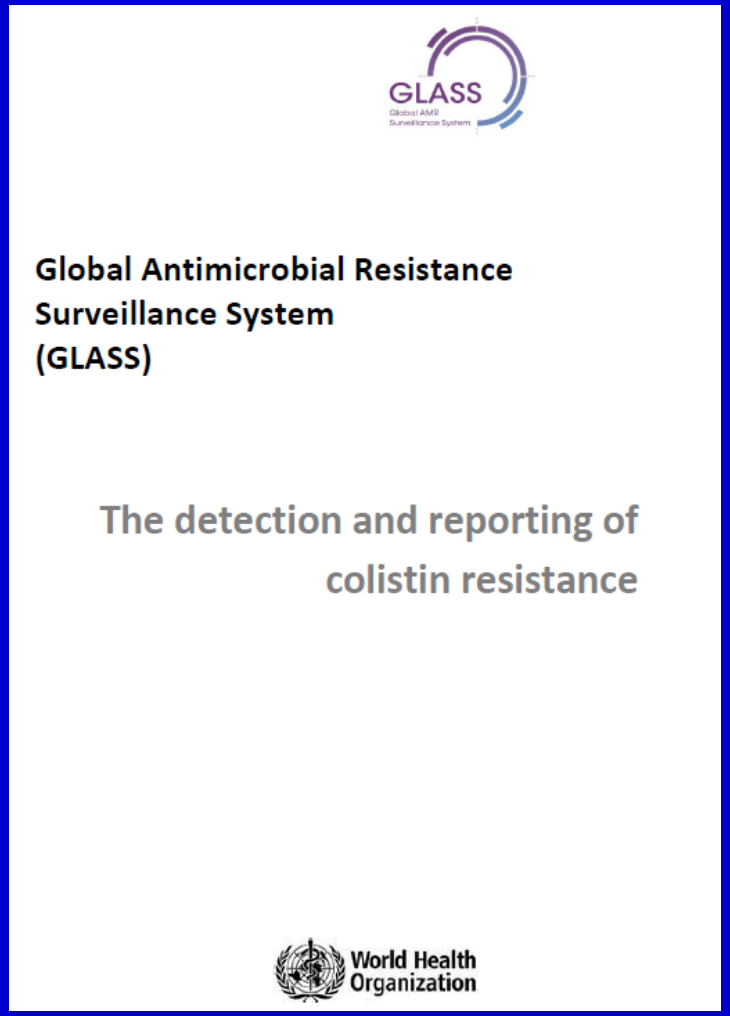
ASM Adelaide 2019

Julie Allerton

Polymyxins

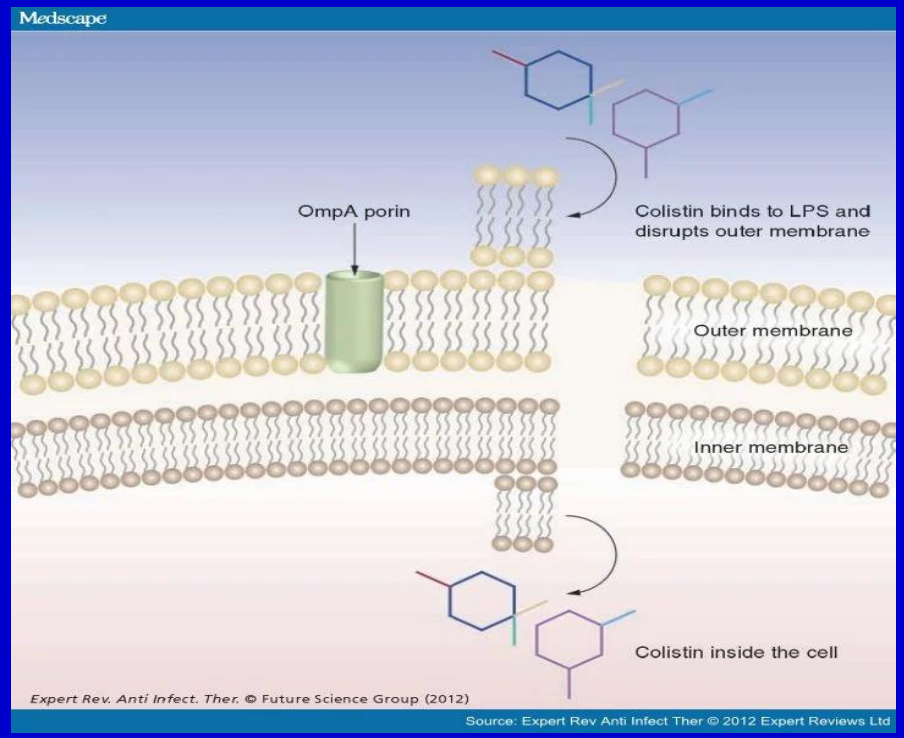
- Increasing need to treat MDR infections
- Combination therapy
  - Carbapenem/carbapenem
  - Carbapenem/polymyxin
  - Carbapenem/tigecycline

WHO Recommendations



Polymyxin family

- Narrow spectrum
- Targets outer membrane
- Polymyxin B – CDS
- Polymyxin E (colistin
  - CLSI
  - EUCAST



Spectrum of Activity

Active	No activity
Escherichia coli	Proteus spp
Enterobacter spp	Morganella morganii
Klebsiella spp	Providencia spp
Citrobacter spp	Serratia marcescens
Pseudomonas aeruginosa	Pseudomonas mallei & Burkholderia cepacia
Stenotrophomonas maltophilia	Chromobacterium spp, Brucella, Leigonella, Campylobacter & V.cholerae
Acinetobacter baumannii	Gram-negative cocci (Neisseria spp)
Salmonella spp	Gram-positive bacteria
Shigella spp	Anaerobic bacteria

Agar dilution

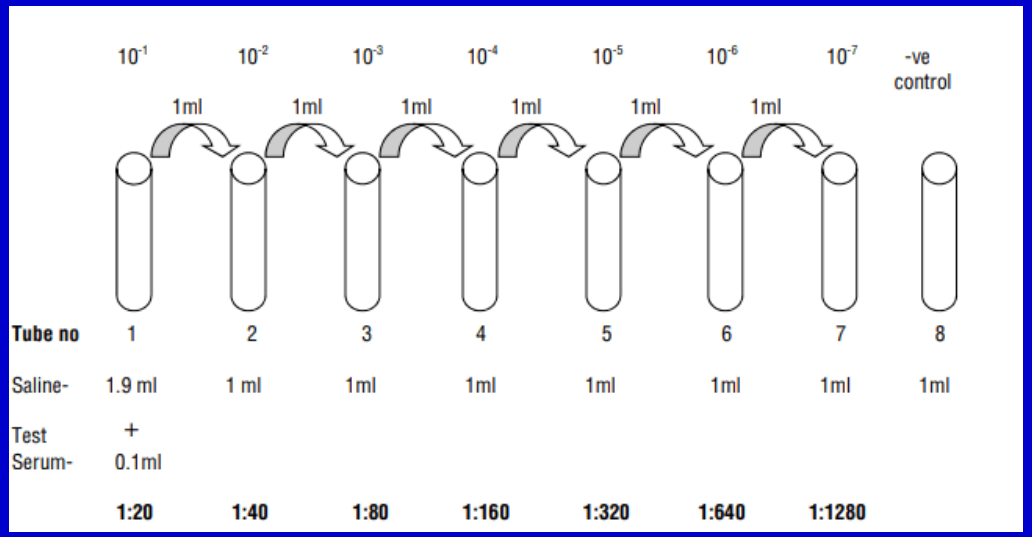
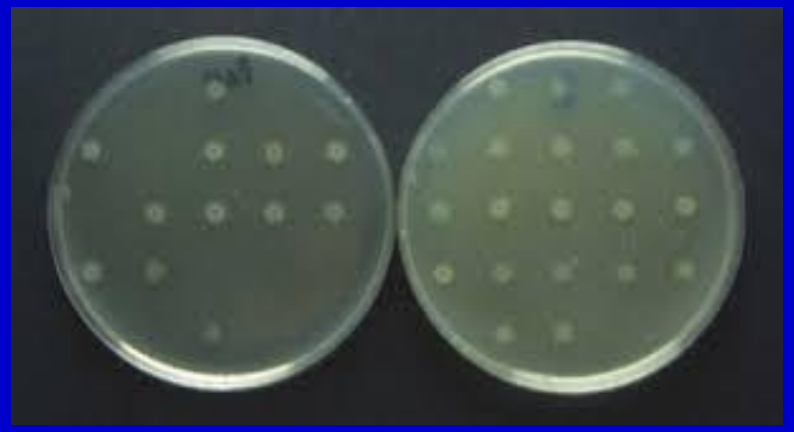
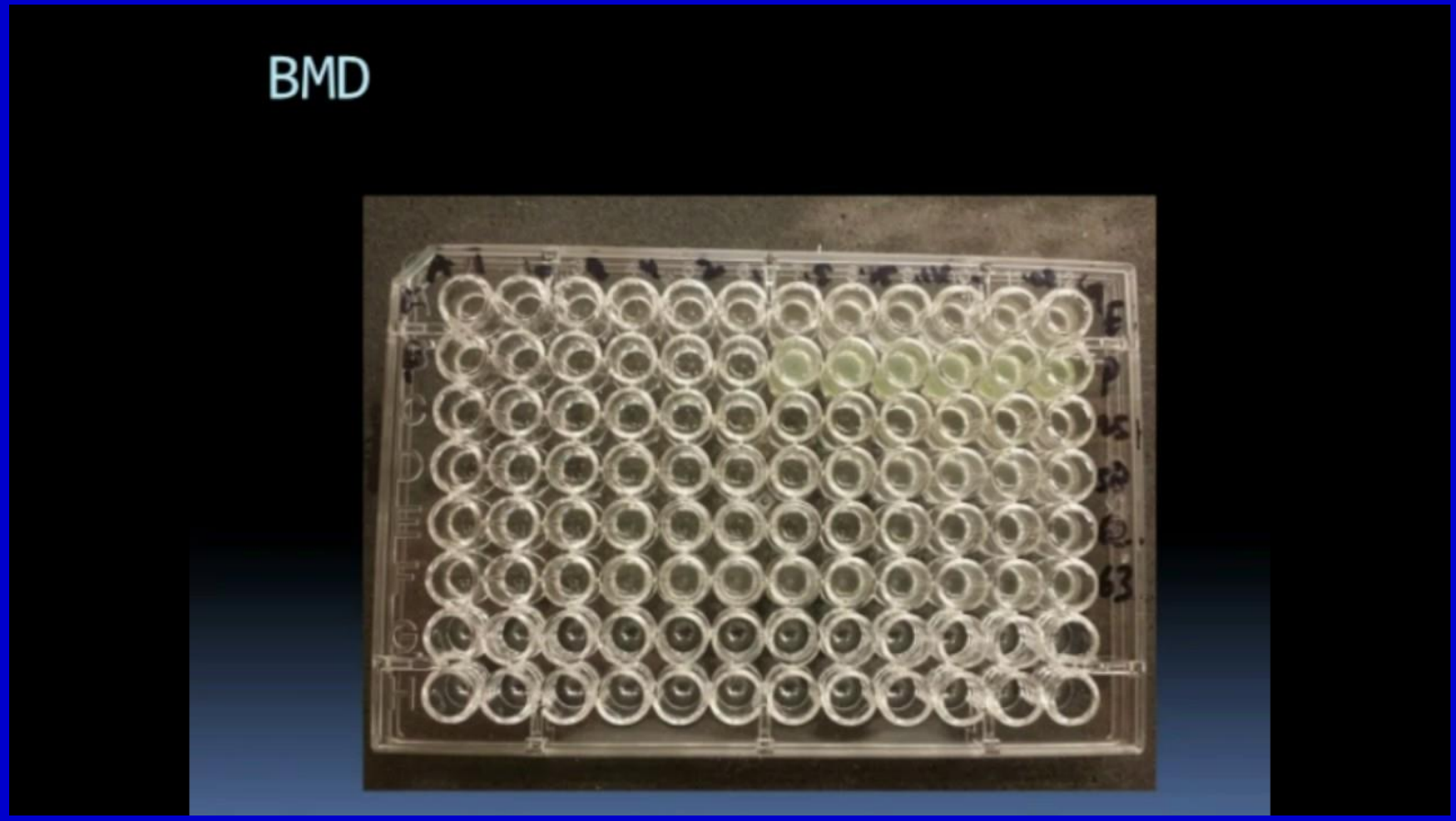


Figure 2.1 ■ **Replicator for agar dilution.** (See Color Plate in the front of the book.)



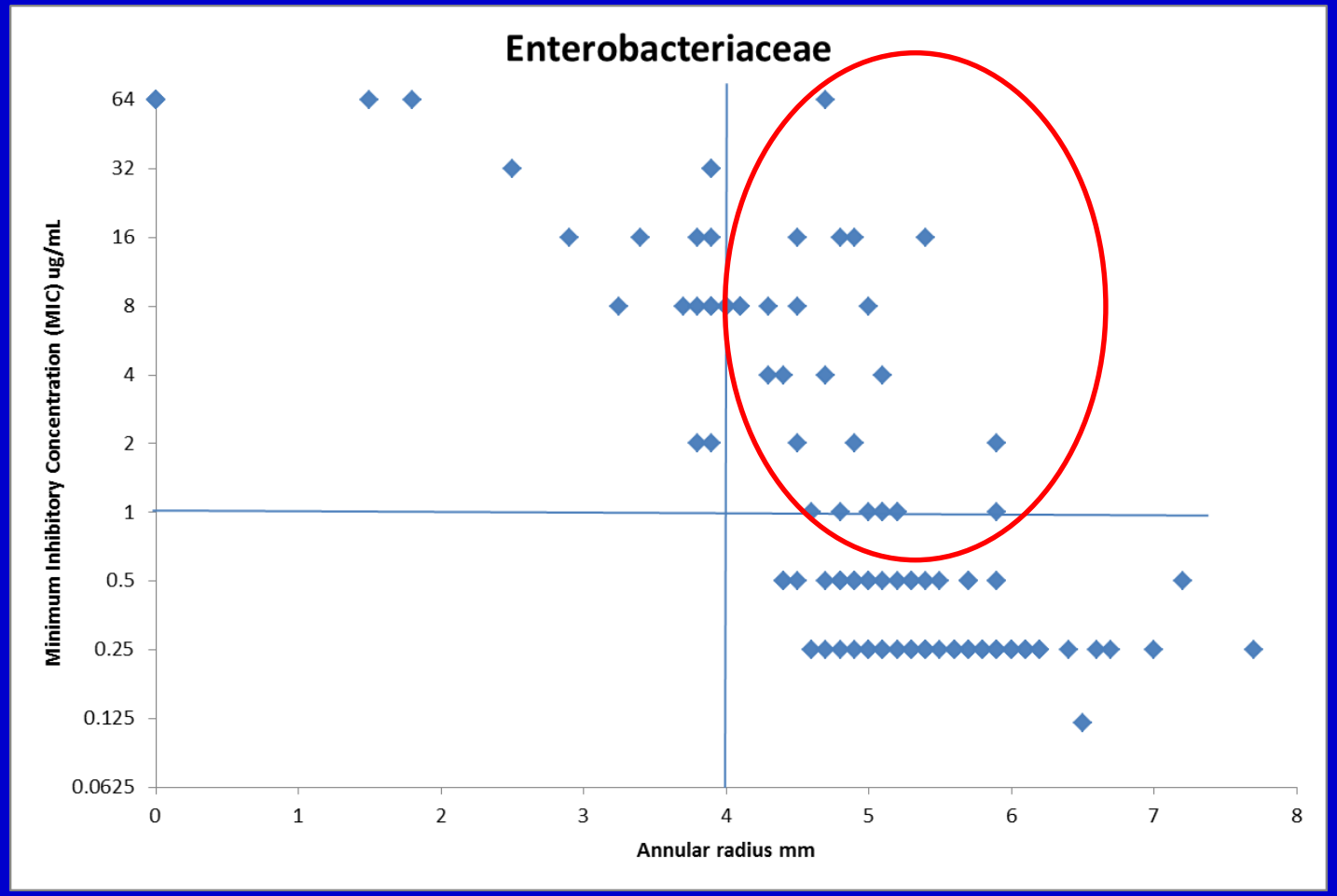
Broth Micro Dilution (BMD)



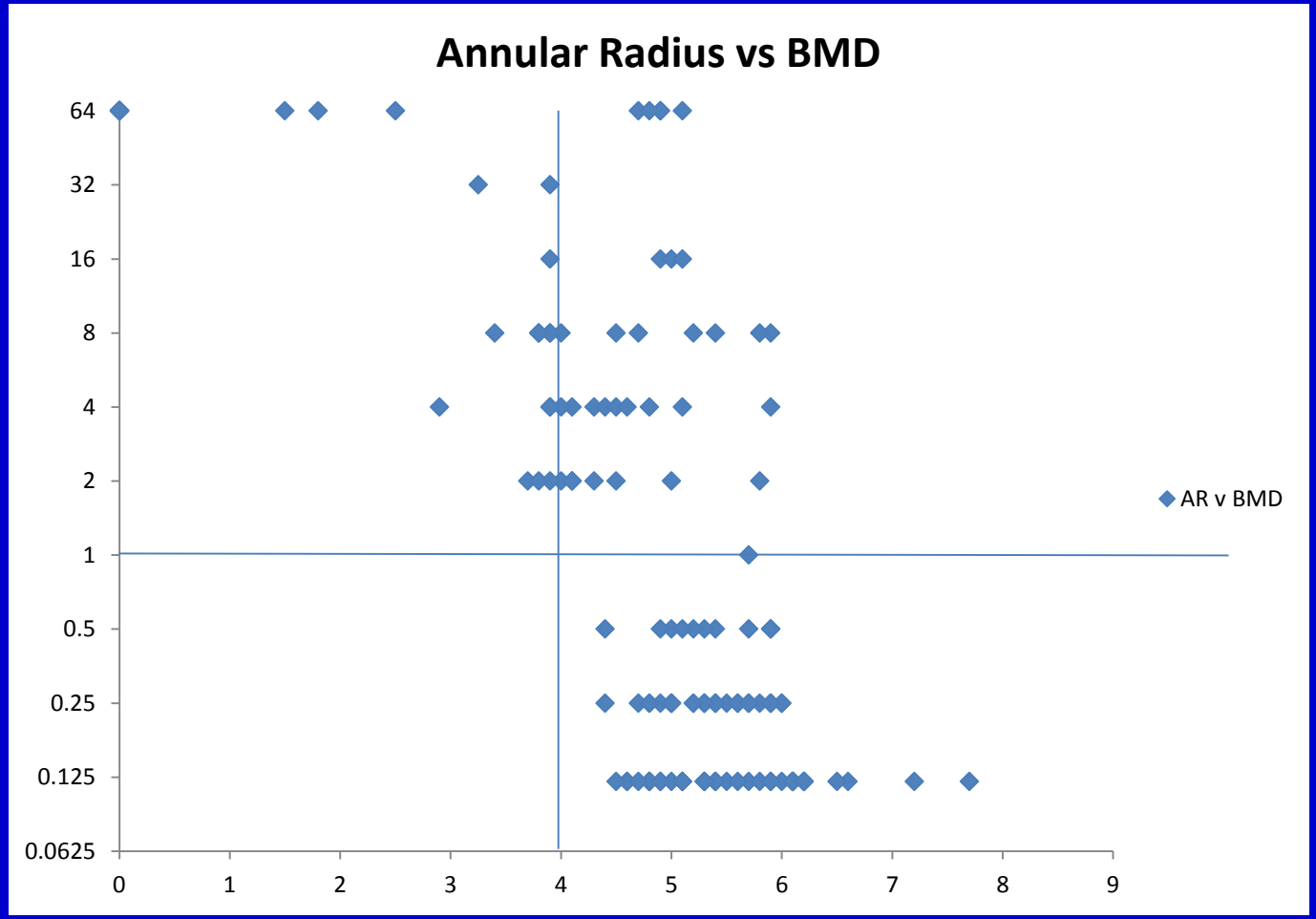
Known Problems

- Slow and poor diffusion through agar
- Varied mechanisms of resistance
- Lack of clinical studies
- ?optimal breakpoint/dosing regime
- Impact of subculture

Enterobacteriaceae



Annular Radius vs BMD

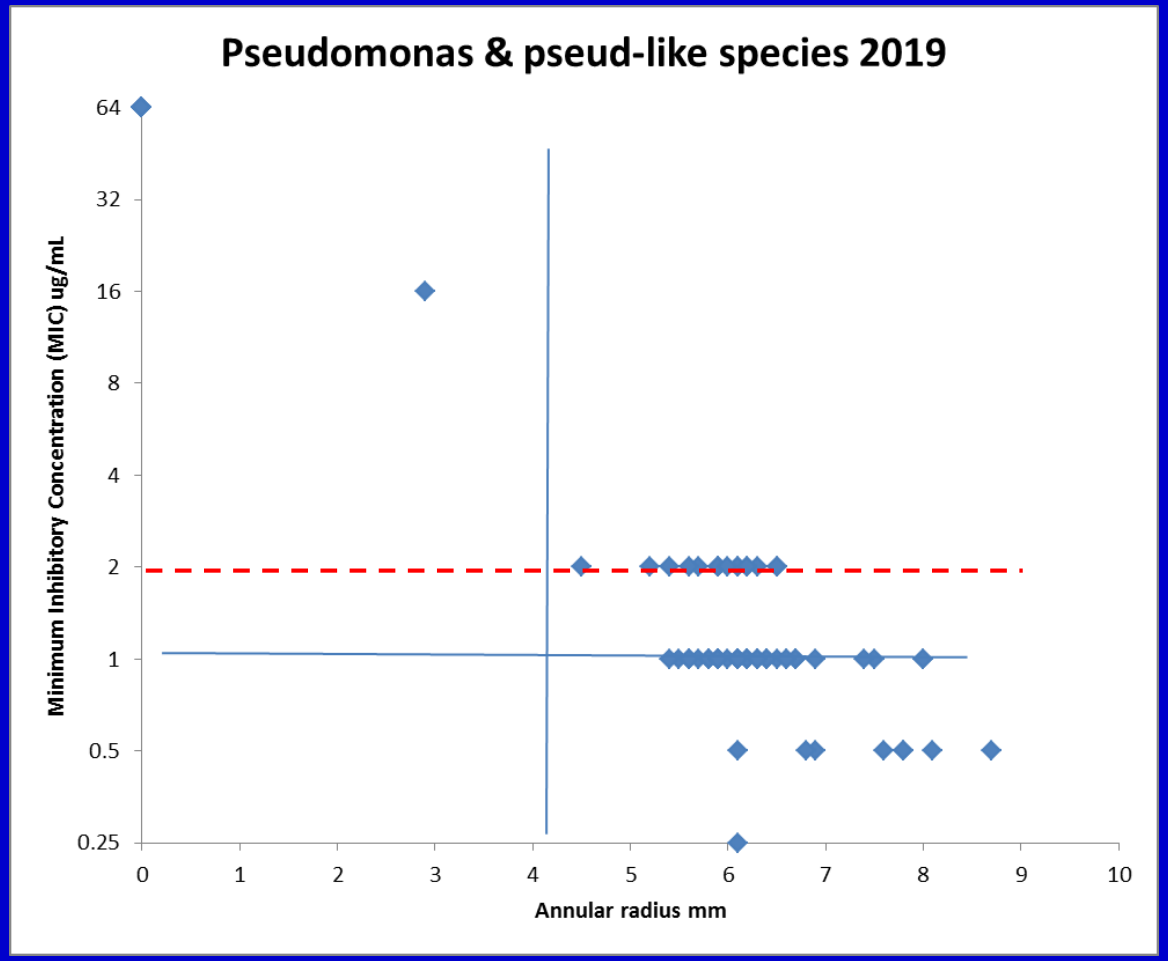


Outliers

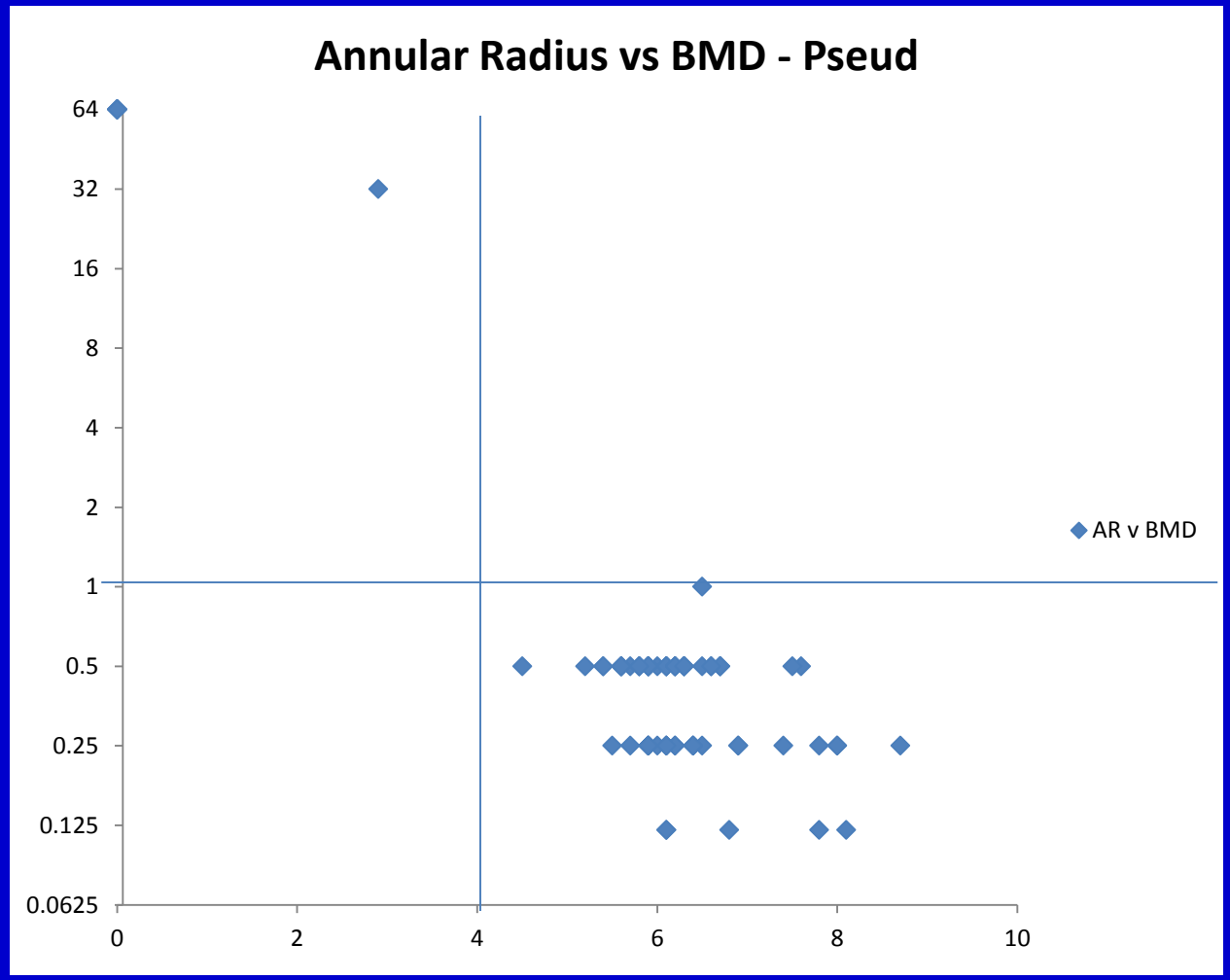
* mcr-1 carrying

- *E. coli* strain
- * annual radius 4mm
- * MIC = 8 ug/mL

Pseudomonas



Annular Radius vs BMD



Current Break Point

- CDS ≤ 1 ug/mL

Enterobacteriaceae and Pseudomonas

- CLSI ≤ 2 ug/mL

Pseudomonas ONLY

- EUCAST ≤ 2 ug/mL

Pseudomonas, Enterobacterales, Acinetobacter

New Break Point

- Problems
  - Lack of clinical studies
  - *in vitro* studies published with few resistant isolates
  - optimized dosage regimes are not known
  - Lack of PK data for IV administration of PB
  - ?adaptive resistance

Unanswered Questions

- Composition of powder used in MIC
- Adsorption by plastics
- “skip wells”
- Storage and subculture of isolates

Alternatives

- Molecular
- E-test
- Vitek 2
- Phoenix
- Sensititre
- Microscan
- Rapid Polymyxin NP

Conclusion

- No conclusion!
- 300 u PB disc still OK for Pseudomonas
- ?use of enzymatic tests for “susceptible” Enterobacteriaceae
- Refer to reference laboratory

Contact Us

- http://cdstest.net
- NSWPATH-SealsCDS@health.nsw.gov.au
- The CDS Reference Laboratory

Department of Microbiology

NSW Health Pathology

Level 3 Clinical Services Building

St George Hospital

Kogarah, NSW 2217

Acknowledgements

Sincere thanks to

- Syd Bell, Dianne Rafferty and Pratibha James for their support and collaboration
- Management and staff in the Microbiology Laboratory at NSWHP

  St George Hospital
- CDS Users group

Carbapenem testing by CDS : An overview

ASM Adelaide 2019

Pratibha James

Key features

- Carbapenems

  Spectrum of activity

  Resistance mechanisms

  Susceptibility testing:

  - Meropenem
  - Imipenem
- CDS Performance in UKNEQAS, an external QAP for Meropenem

Need for accurate carbapenem susceptibility testing

- Antimicrobial resistance on the rise and essential to have an accurate susceptibility testing to maximise therapeutic options.
- Carbapenems are usually the last resort drugs for treating MDR organisms necessitating optimal susceptibility testing.

Carbapenems: Spectrum of activity

Broad spectrum :

- Gram-negative organisms (including beta-lactamase producing H. influenzae and N. gonorrhoeae, the Enterobacteriaceae, and P. aeruginosa), including those that produce extended-spectrum beta-lactamases
- Anaerobes (including B. fragilis)
- Gram-positive organisms (including Enterococcus and Listeria)

Carbapenems: Spectrum of activity

- Imipenem and Meropenem have a similar spectrum (Exception being Proteus spp, Providencia spp & Morganella spp which are intrinsically resistant to Imipenem).

Carbapenems: Spectrum of activity

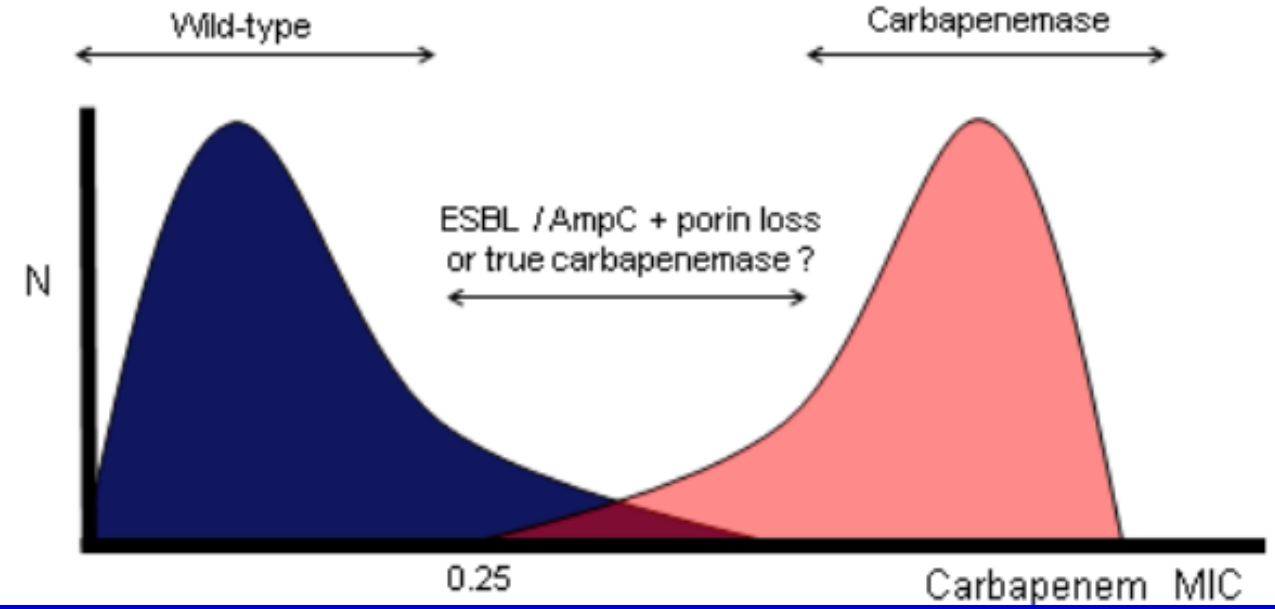
- Imipenem and Meropenem have a similar spectrum (Exception being Proteus spp, Providencia spp & Morganella spp which are intrinsically resistant to Imipenem).
- Ertapenem — Newer carbapenem with a narrower spectrum of activity than imipenem or meropenem. It is active against most Enterobacteriaceae and anaerobes but less active than the other carbapenems for P. aeruginosa, Acinetobacter, and Gram positive bacteria, particularly enterococci and penicillin-resistant pneumococci.

Carbapenem resistance

- Combination of mechanisms can be responsible for reduced susceptibility to carbapenems :
  a) Permeability defects (porin mutation, upregulation of efflux pumps)
  b) Production of β-lactamases with weak carbapenemase activity (Ambler class A, ESBLs or Ambler class C, AmpC cephalosporinases, CTX-M-15, CMY-2)
- Carbapenemases (Ambler class A,B or D)

Public Health England

The problem with spotting the carbapenemase producers



Susceptibility testing

- Ertapenem- high sensitivity but low specificity (not recommended for routine use)
- Imipenem- Not recommended for use as a stand-alone screening test compound because relatively poor at separating wild-type organisms and carbapenemase producers.
- Meropenem- best compromise between sensitivity and specificity in terms of detecting carbapenemase producers.

Utility of Imipenem in CDS

- For detecting inducible cephalosporinase by placing imipenem disc adjacent to cefotaxime/cefotetan.

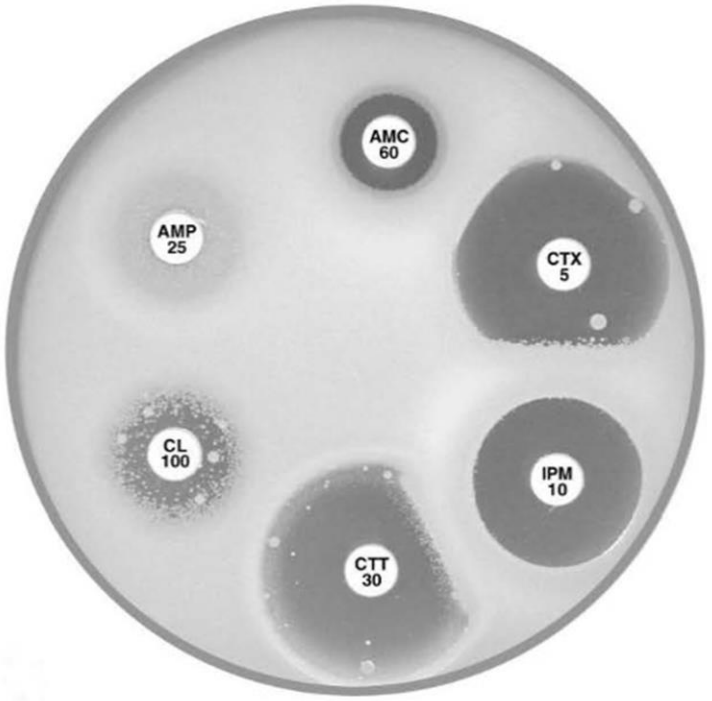
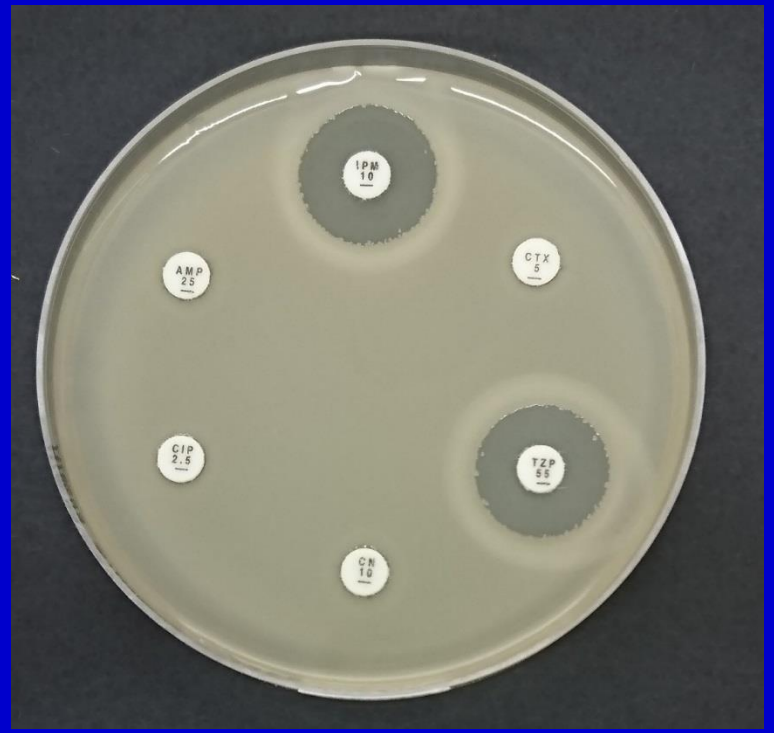


Plate 12.12.A *Enterobacter cloacae* with AmpC β-lactamase
The flattened inhibitory zone around imipenem (IPM 10) adjacent to cefotaxime/cefotetan (CTX 5, CTT 30) demonstrates the presence of a basal inducible cephalosporinase.

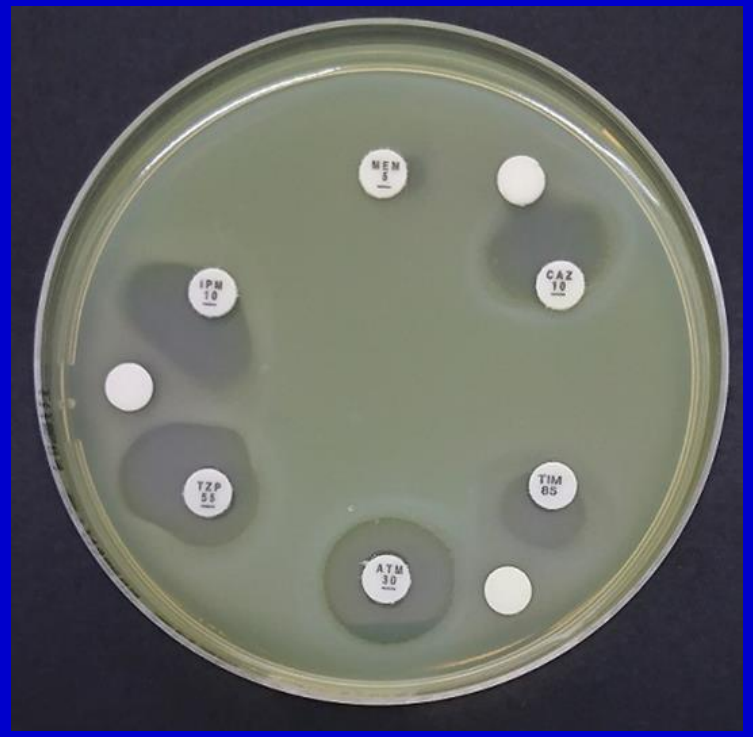
Why use Meropenem?

- Meropenem has replaced Imipenem as the therapeutic agent in Australia.
- Meropenem cannot be substituted with Imipenem for testing as some members of Enterobacteriaceae family are intrinsically resistant to Imipenem.

K pneumoniae CTX-1 & carbapenemase



Detection of MBL

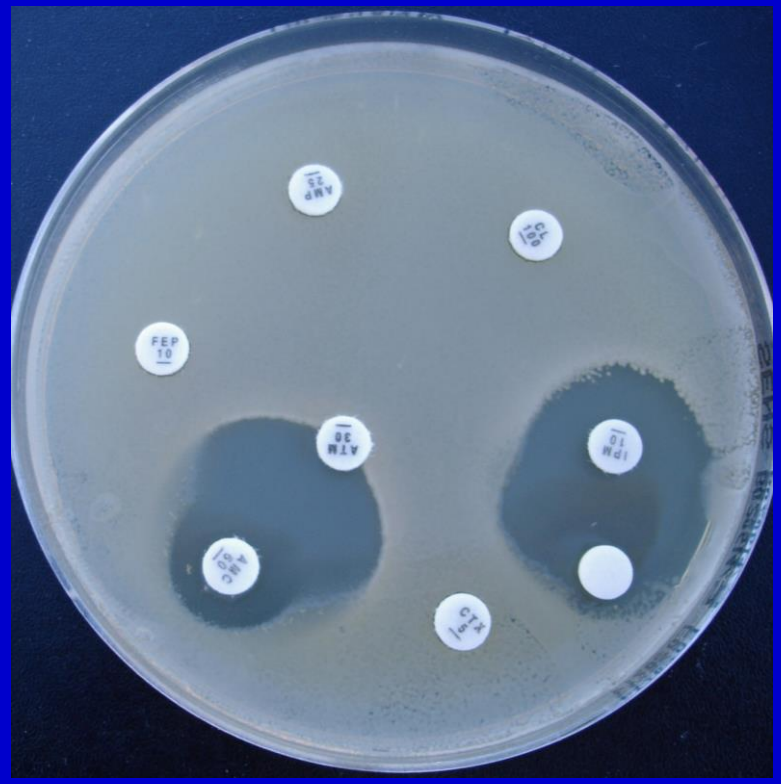


Synergy between an EDTA disc (blank disc) placed next to imipenem (IMP 10), tazocin (TZP 55), Timentin (TIM 85), ceftazidime (CAZ10), S/aztreonam (ATM30)

Detection of MBL

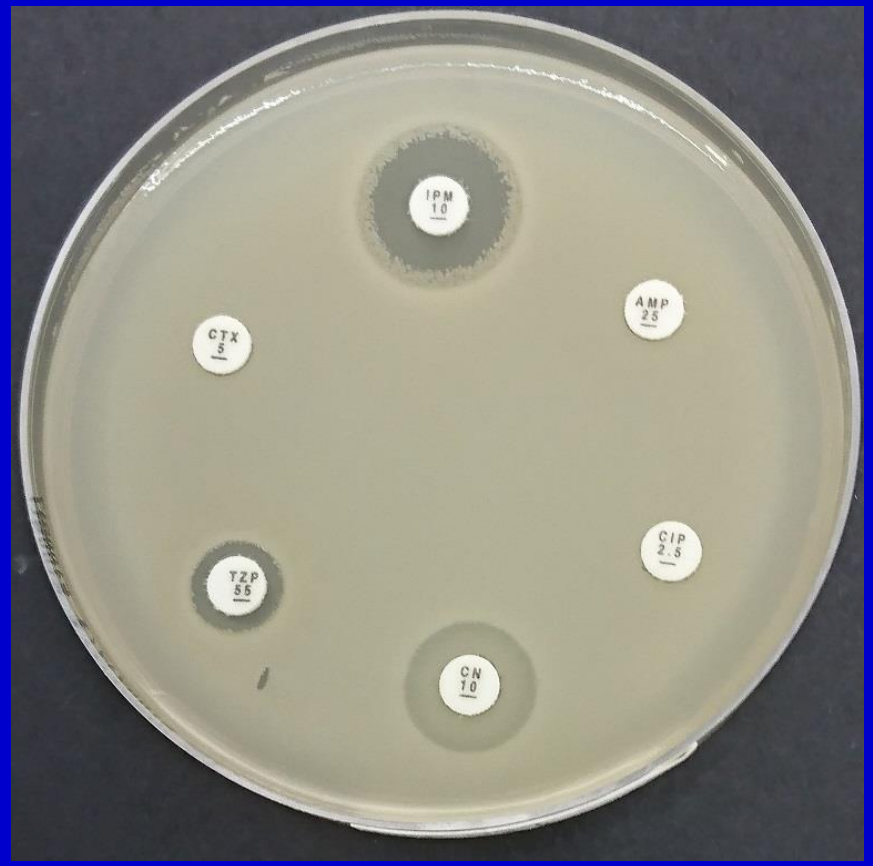
Synergy between an EDTA disc (blank) placed next to cefotaxime (CTX 5)/ imipenem (IPM 10)/ cefepime (FEP 10)/ Augmentin (AMC 60) discs

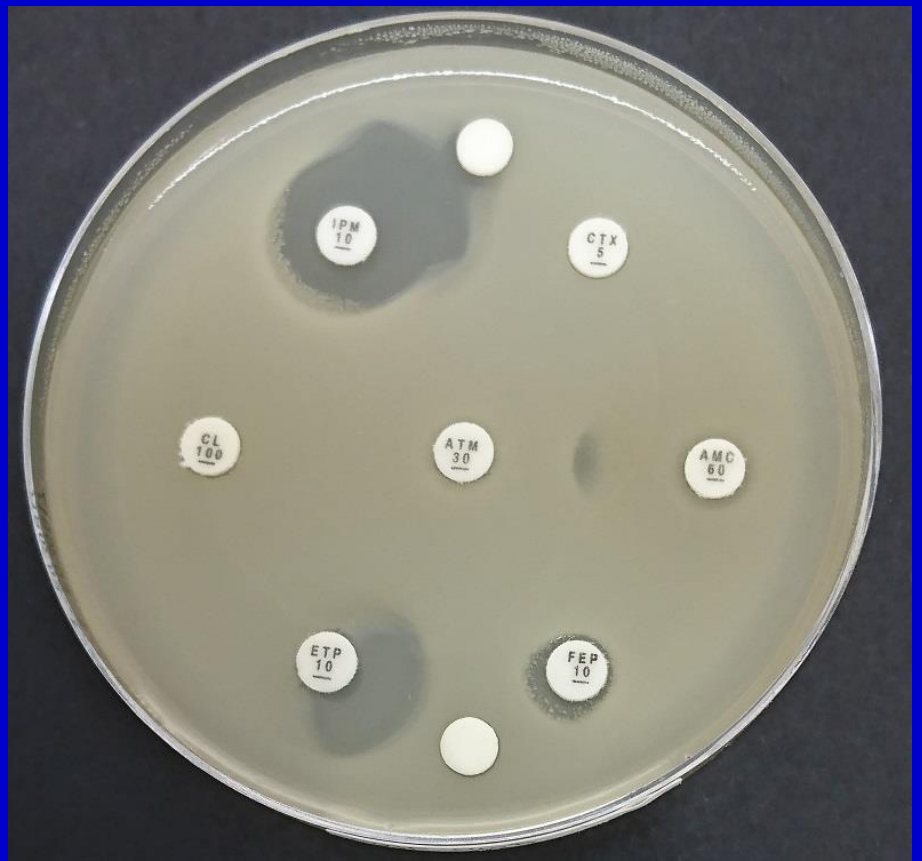
E coli bla-NDM & ESBL



Synergy between an EDTA disc (blank disc) placed next to imipenem (IMP 10), synergy between Augmentin (AMC 60) Aztreonam (ATM30)

K pneumoniae MBL(NDM) & CTX-M





KPC

E. coli Oxa-48


AMP 25
MEM 10
CI 196
IPM 10
AMC 60
CTX 5


AMC 60
ATM 30
FEP 10
TZP 55
ETP 10

Oxa-48 and Oxa-181

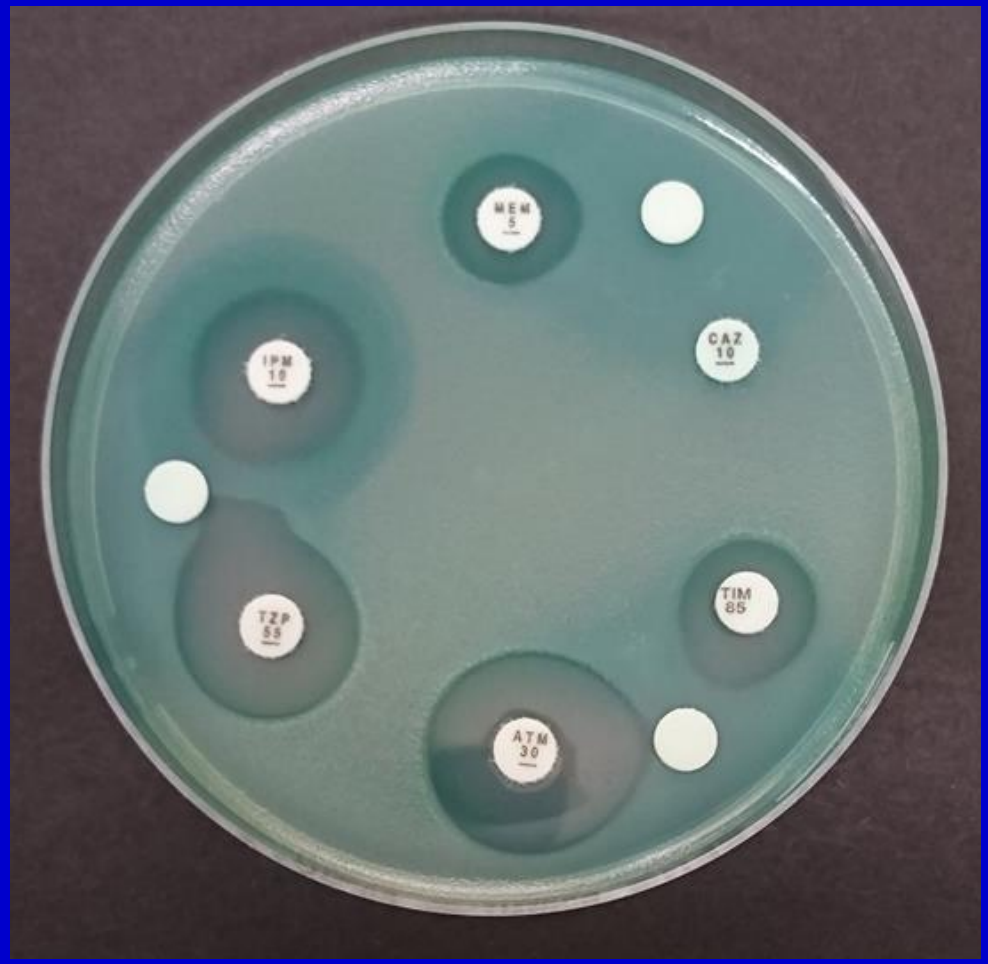
- No definitive Phenotypic test only Presumptive ID
- Imipenem annular radius (AR)<6mm or 6-7 mm with mutant colonies at zone edge

  Plus
- Resistant to Augmentin & Tazocin

  Plus
- Cefotaxime/Ceftriaxone & Cefepime AR > carbapenems
- Perform further confirmatory tests

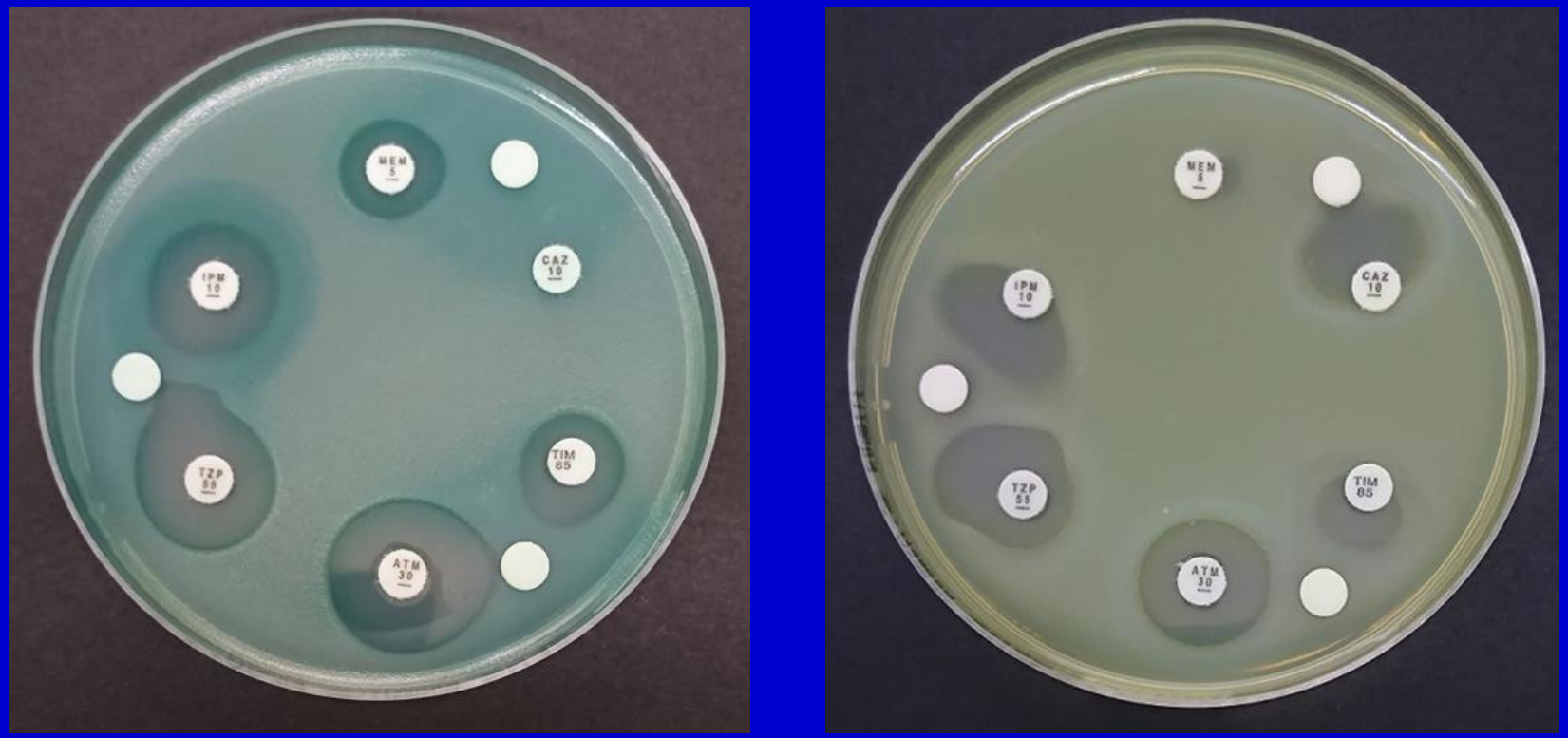
Pseudomonas aeruginosa highly resistant to all β-lactams ?MBL

Detection of MBL:
Non-specific synergy – Not MBL

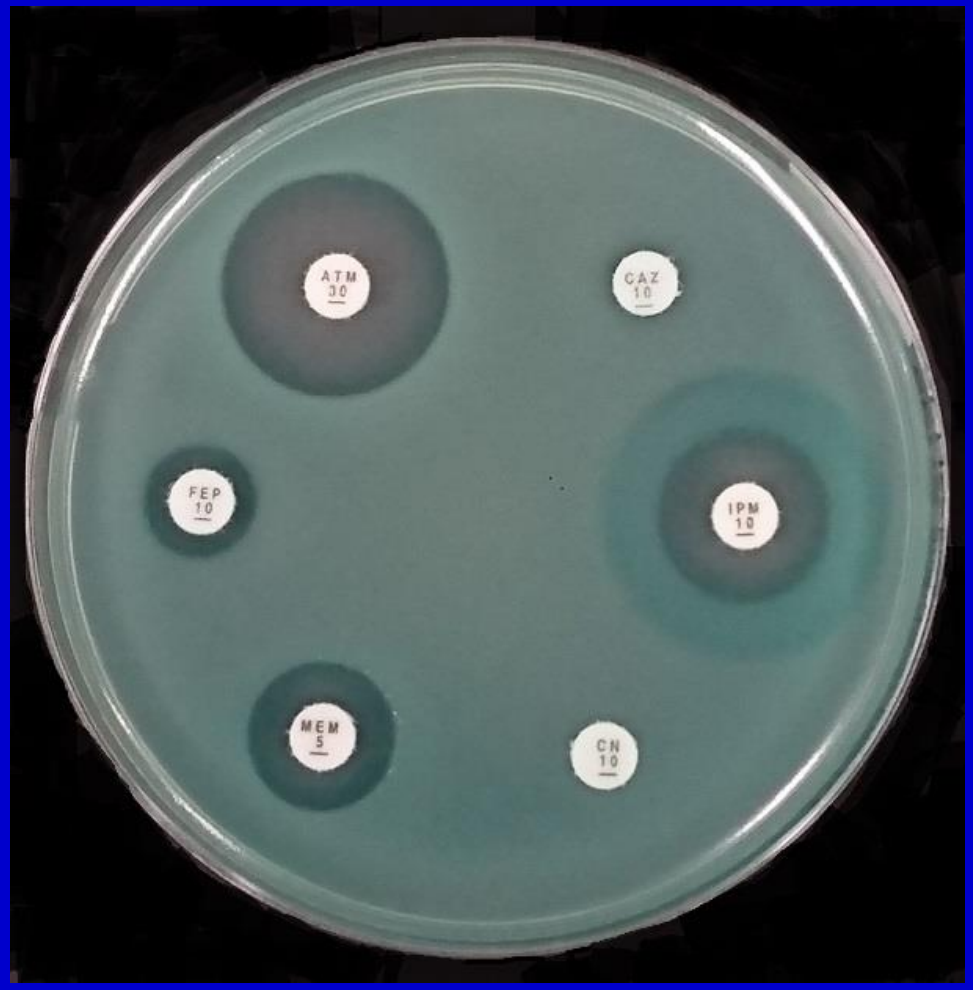


Comparison of EDTA results

Non-specific synergy(left) and specific synergy in MBL positive strain (right)

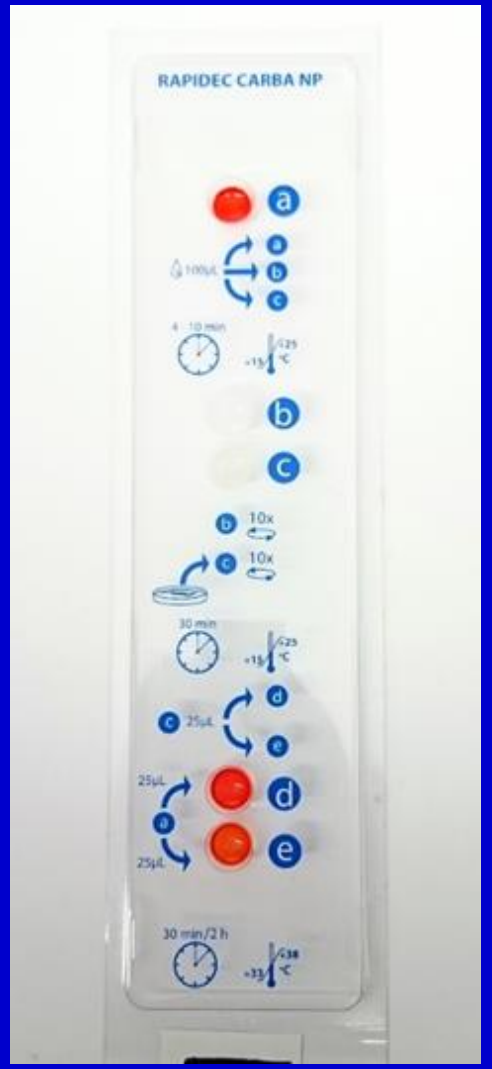


Ps species *bla*-GES

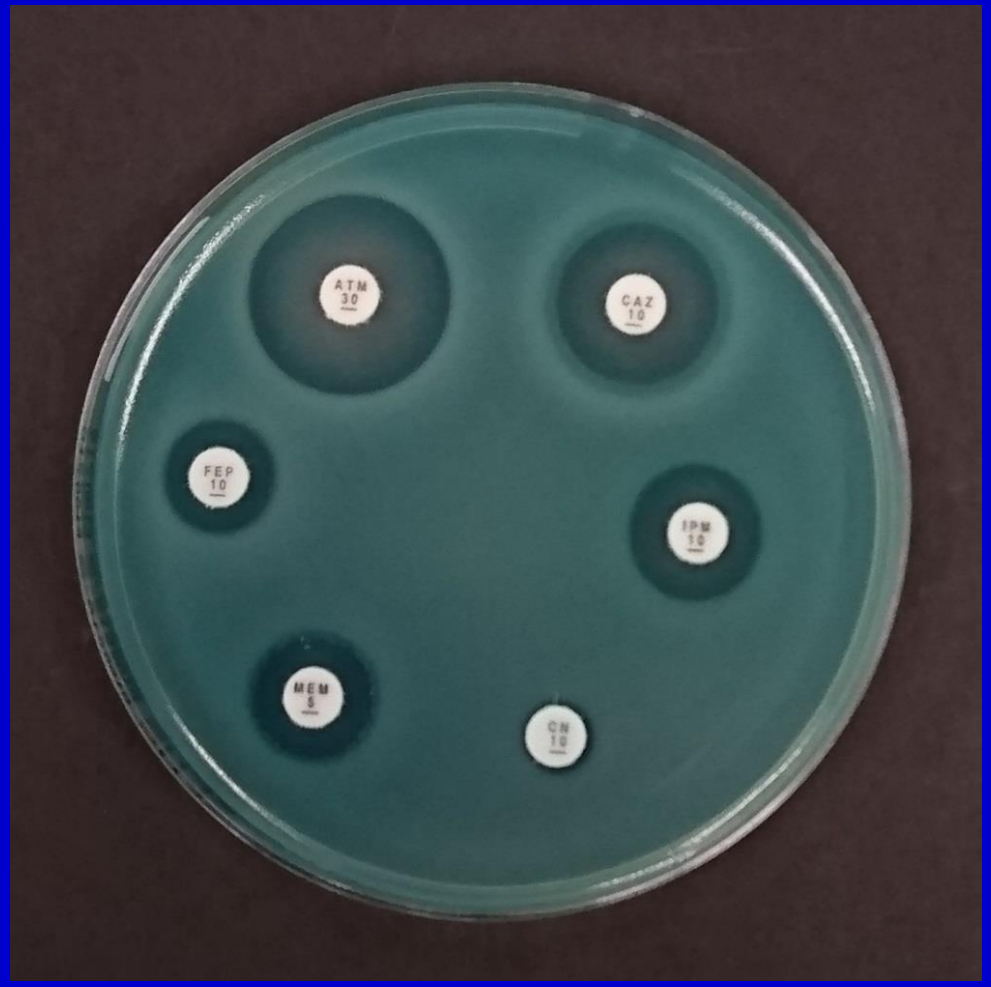


Ps species *bla*-GES

- CarbaNP negative
- Confirm with molecular testing



Ps species *bla*-VIM



CDS performance in UKNEQAS for Meropenem

Between September 2016 to date

27 isolates tested

Major error- Reporting resistant isolate as susceptible

Minor error- Reporting susceptible isolate as resistant

Results:

Enterobacteriaceae- 19 with two minor errors

Acinetobacter spp- 2 with no errors

Pseudomonas spp- 6 with no errors

Acknowledgements

Sincere Thanks to:

- Prof Sydney Bell
- Dianne Rafferty
- Julie Allerton

Updates in CDS

- Comprehensive summary can be found in “What’s New” Newsletter 45 on the website: http://cdstest.net/
- Chapter 8 – Application of the CDS to Unusual organisms
- Chapter 11.1 – Calibration of Ceftolozane-tazobactam
- Chapter 11.1 – Polymyxin testing
- Chapter 11.2 – Update Ofloxacin no longer for veterinary use