Welcome to the 34th

ASM CDS Workshop

Perth 2016

Dianne Rafferty

SEALS excellence in diagnosis research teaching

NSW GOVERNMENT Health Pathology

CDS Workshop Topics

- Dianne Rafferty - Current CDS items

  - Reconciliation of Agar MIC and Broth MIC

- Julie Allerton – The Current Role of Phenotypic Antibiotic Susceptibility Testing in Enterobacteriaceae

- Peter Newton – *Streptococcus* spp. & *Haemophilus* spp. with decreased β-lactam susceptibility: CDS reporting.

  - Recognition of OXA-48 like CPE with the CDS Method – a case study

What's been happening with the CDS ?

- Julie Allerton has come on board
- Email address change
  - NSWPATH-SealsCDS@health.nsw.gov.au
  - Dianne.Rafferty@health.nsw.gov.au
  - Julie.Allerton@health.nsw.gov.au
- Website address
  - cdstest.net/

What's been happening with the CDS

- Formation of ASM SIG
- Evaluation of Timentin discs
- Pradofloxacin for Veterinary use
- Reconciliation of Agar MICs and broth MICs

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MAST Timentin 85µg Disc

- *Ps aeruginosa NCTC 10662*
- Evaluation over 29 days
- Strict adherence to the manufacturer's guidelines required
- Potency maintained

What's been happening with the CDS

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- Pradofloxacin for Veterinary use
- Reconciliation of Agar MICs and broth MICs

Pradofloxacin

- 3rd Generation fluoroquinolone
- Used in the treatment of skin infections and UTI in cats
- Active against both Gram positive and Gram negative bacteria

Pradofloxacin Surrogate Disc Testing

Isolates n = 58	Ciprofloxacin	Moxifloxacin	Pradofloxacin
B bronchiseptia (6)	S/6	S/6	S/6
Pasteurella multocida (7)	S/7	S/7	S/7
Pasteurella sp (9)	R/1 S/8	S/9	S/9
S aureus (10)	R/4 S/6	S/10	S/10
S pseudintermedius (10)	R/8 S/2	R/6 S/4	R/3 S/7
E coli (10)	S/9 R/1	S/9 R/1	S/9 R/1
Enterococcus sp (6)	S/6	S/3 R/3	S/3 R/3

Pradofloxacin

Moxifloxacin 2.5µg can be used as a surrogate disc for Pradofloxacin

What's been happening with the CDS

- Formation of ASM SIG
- Evaluation of Timentin discs
- Pradofloxacin for Veterinary use
- Reconciliation of Agar MICs and broth MICs

What is an MIC?

- Defined as the lowest level of an antibiotic required to visibly inhibit the growth of a specific organism after overnight incubation
- All methods have an inherent ±1 dilution error
- 1971 Ericsson & Sherris

Why Agar Dilution is recognised as the 'Gold Standard'?

- Better reproducibility
- Simple to perform
- Ability to detect contamination
- Application with a broader range of organisms
- Ability to detect heterogeneity

ISO 20776 -1

- Broth and Broth micro-dilution MIC
- *In-vitro* activity of antimicrobial agents against rapidly growing aerobic bacteria only
- Commercial Broth micro-titer MIC plates not ISO compliant
- MIC for QC strains

Reconciliation of MIC methods

- Putting the ISO furphy to rest
- Reconciliation project
- Gold Standard (Agar dilution) vs ISO 20776-1 (Broth micro-dilution)
- QC reference strains

Reconciliation of MIC methods

- Inoculum of Agar MIC 10^4cfu/spot
- Inoculum of Broth MIC 10^5 cfu/mL
- Inherent ±1 dilution factor all MIC methods

Reconciliation Project Preliminary Results

S aureus NCTC 29213	Agar MIC - CDS (mg/L)	Agar MIC – MH (mg/L)	Broth MIC (mg/L)	ISO Reference Range (mg/L)
Ampicillin	1.0	2.0	1.0	0.5-2.0
Benzylpenicillin	0.5	0.5-4.0	0.25-1.0	0.25-2.0
Ciprofloxacin	0.5	0.5	0.5	0.12-0.5
Moxifloxacin	0.06	0.06	0.06	0.015-0.12

Note: CDS = Sensitiest Agar; MH = Mueller Hinton Agar

Note: Staph aureus NCTC 29213 is weak β-lact positive

Reconciliation Project Preliminary Results

E coli NCTC 25922	Agar MIC - CDS (mg/L)	Agar MIC – MH (mg/L)	Broth MIC (mg/L)	ISO Reference Range (mg/L)
Ampicillin	8.0	16.0	4.0	2.0-8.0
Ciprofloxacin	0.015	0.015	0.008	0.004-0.015
Ertapenem	0.015	0.008	0.015	0.004-0.015
Impenem	0.12	0.25	0.25	0.06-0.25
Moxifloxacin	0.06	0.06	0.008	0.008-0.06
Meropenem	0.015	0.015	0.015	0.008-0.06

Note: CDS = Sensitiest Agar; MH = Mueller Hinton Agar

Reconciliation of Agar MICs and broth MICs

- Preliminary results indicate good correlation between all methods
- Further investigation is ongoing

References

1. **Silley, P., Bernd, S. *et al*.** 2012. Bacterial properties of pradofloxacin against veterinary pathogens. Veterinary Microbiology 157**:106-111**.

2. **Ericsson, H.M. & Sherris, J.C.. 1971. Antibiotic Sensitivity Testing *Acta Path Microbiology.* Scand. 217:Suppl.**

3. **Bell, S.M. 1975. The CDS disc method of antibiotic sensitivity testing (Calibrated Dichotomous Sensitivity Test.) Pathology. 7:No.4. Suppl. 1-48.**

The Current Role Of Phenotypic Antibiotic Susceptibility Testing In Enterobacteriaceae

ASM 2016

Julie Allerton

Overview

- Aims of susceptibility testing
- Detection by molecular and traditional disc methods
- Review methods of identifying resistance

The Aims Of Antimicrobial Susceptibility Testing (AST)

- Determine antibiotic susceptibility
- Demonstration of resistance
- Produce timely and accurate results

Definition of Phenotype

- Observable characteristic
- Influenced by genetics and environment
- Useful for selecting appropriate antimicrobial agents for treatment

Disc Method Of AST

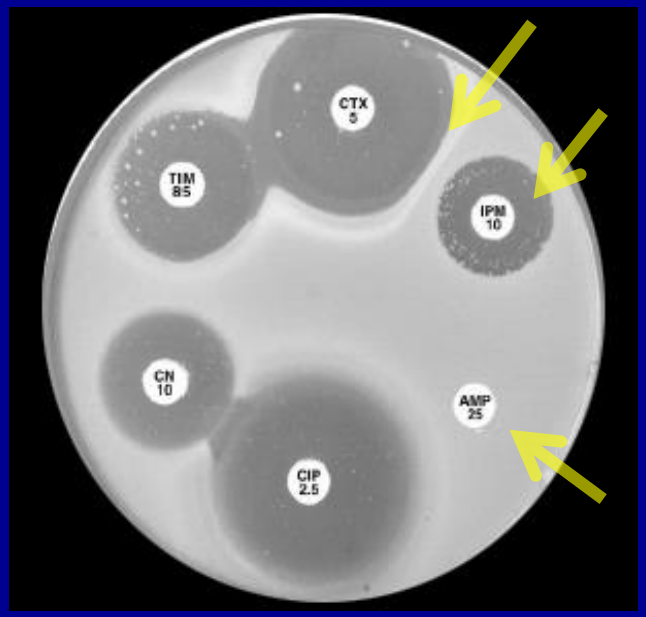
- Well standardised
- Calibrated to ‘Gold Standard’ (MIC)
- Straightforward to perform
- Reproducible
- Inexpensive

The Detection Of Resistance By Disc Testing

- Measurement of zone size
- Inhibitory zone morphology
- Supplementary use of other agents

Phenotypic Resistance

- No zone or zone <6mm*
- Morphology of Zone
- Variants, or mutants, within inhibitory zone
- Sometimes not obvious (EEC group)



*Notable exceptions include Gentamicin, Amikacin, Polymyxin B, Tetracycline and Tobramycin

12.4. Testing and reporting β-lactam antibiotics

Table 12.4.a. A guide to the testing and reporting of β-lactam antibiotics for Gram negative organisms.

Organism	Antibiotic [b,c] AMP	AMC	ATM	CAZ	CXM,CL,CPD CRO,CTX,KZ	CPO FEP	CTT	FOX	IPM,MEM ETP, DOR	PRL	TIM	TZP
Enterobacteriaceae of the EEC group [d]	R	R	R	R	R	T	R	R	T	N [a]	R	R
Serratia marcescens	R	R	T	T	R	T	R	R	T	N [a]	R	T
Aeromonas with A1 cephalosporinase & A2 carbapenemase	R	R	T*	T*	R	T	R	R	R [e]	N [a]	R	R
Aeromonas sobria/veronii with A2 carbapenemase only	R	R	T	T	T	T	T	T	R	N [a]	T	T
Proteus vulgaris / penneri [f]	R	U	T	T	R	T	T	T	T	N [a]	U	U
C. amalonaticus	R	U	R	T	R	T	T	T	T	N [a]	U	U
Enterobacteriaceae with ESBL	R	U	R	R	R	R	T	R	T	N [a]	U	U
Enterobacteriacae with an MBL	R	R	T	R	R	R	R	R	R	N [a]	R	R
Pseudomonas species / *Burkholderia* species [g] / *Chryseobacterium* species	R	R [g]	T	T	R	T	R	R	T	T	T	T
P. aeruginosa with ESBL	R	R	R	R	R	R	R	R	T	R	U	U
P. aeruginosa with an MBL	R	R	T	R	R	R	R	R	R	R	R	R
Stenotrophomonas maltophilia [h]	R	R	R [h]	R [h]	R	R	R	R	R	R [h]	R [h]	R [h]

[a] N = Not calibrated for this species.

[b] R = The isolate is resistant to the antibiotic because it possesses a mechanism of resistance that may not be demonstrated by disc testing.
T = Can be tested and reported.
T*= Can be tested and reported (See section 5.2 on *Aeromonas*).
U = Test isolates from uncomplicated UTI only. Isolates from other sites are considered RESISTANT.

[c]

AMP	ampicillin	CL	cephalexin	DOR	doripenem	KZ	cefazolin
AMC	Augmentin	CPD	cefpodoxime	ETP	ertapenem	MEM	meropenem
ATM	aztreonam	CPO	cefpirome	FEP	cefepime	PRL	piperacillin
CAZ	ceftazidime	CRO	ceftriaxone	FOX	cefoxitin	TIM	Timentin
CXM	cefuroxime	CTT	cefotetan	IPM	imipenem	TZP	Tazocin
CTX	cefotaxime						

[d] *Enterobacter aerogenes, Enterobacter cloacae, Citrobacter freundii.*

[e] *A. caviae* does not possess a carbapenemase (A2) and can be tested against imipenem, meropenem, ertapenem and doripenem.

[f] Isolates with high β-lactamase activity may give no zone around CTX 5 µg but show a "key-hole" effect that may be mistaken as an indication of the presence of an ESBL. However, unlike ESBL producers, they may be susceptible to ceftazidime.

[g] *B. pseudomallei* is usually susceptible to Augmentin and can be tested against this antibiotic.

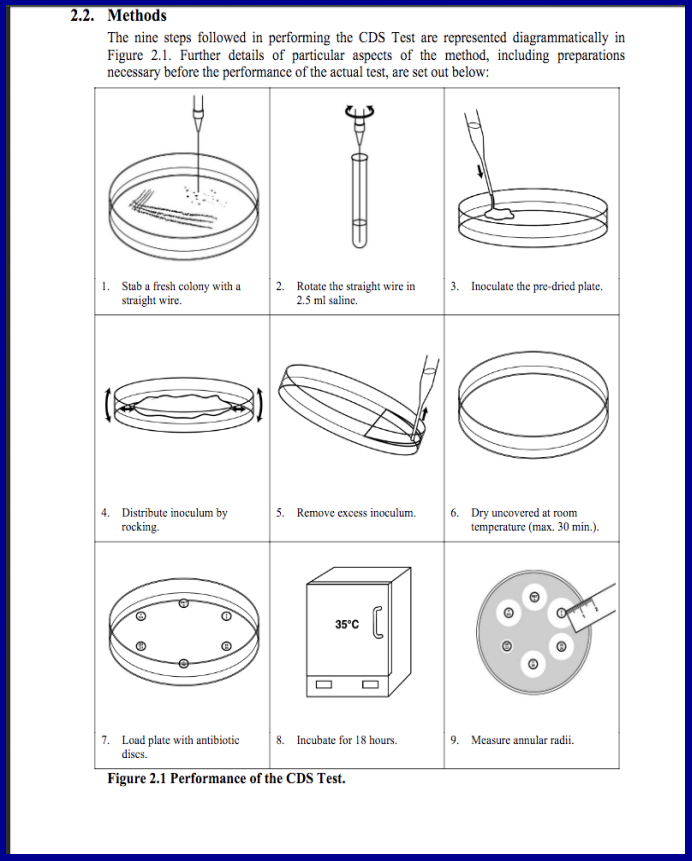
[h] See Section 5.11 for notes on testing *S. maltophilia* against these and other antibiotics.

Defining Resistance By Gene Detection

- More rapid result than disc diffusion
- Limited utility
- High cost
- Possibility of over or under estimating resistance

Advantages Of CDS Method In Detection Of Resistance

- Higher inoculum of 10^7 cfu/mL
- Lower potency antibiotics
- Strategic positioning of antibiotic discs
- Access to CDS reference laboratory



2.2. Methods

The nine steps followed in performing the CDS Test are represented diagrammatically in Figure 2.1. Further details of particular aspects of the method, including preparations necessary before the performance of the actual test, are set out below:

1. Stab a fresh colony with a straight wire.
2. Rotate the straight wire in 2.5 ml saline.
3. Inoculate the pre-dried plate.
4. Distribute inoculum by rocking.
5. Remove excess inoculum.
6. Dry uncovered at room temperature (max. 30 min.).
7. Load plate with antibiotic discs.
8. Incubate for 18 hours.
9. Measure annular radii.

Figure 2.1 Performance of the CDS Test.

Characteristics Of Extended Spectrum β-lactamases

- Plasmid mediated
- R/ Cephalosporins
- S/ Augmentin
- S/ Cephamycins
  (cefoxitin and cefotetan)
- S/ Imipenem

Types Of AmpC

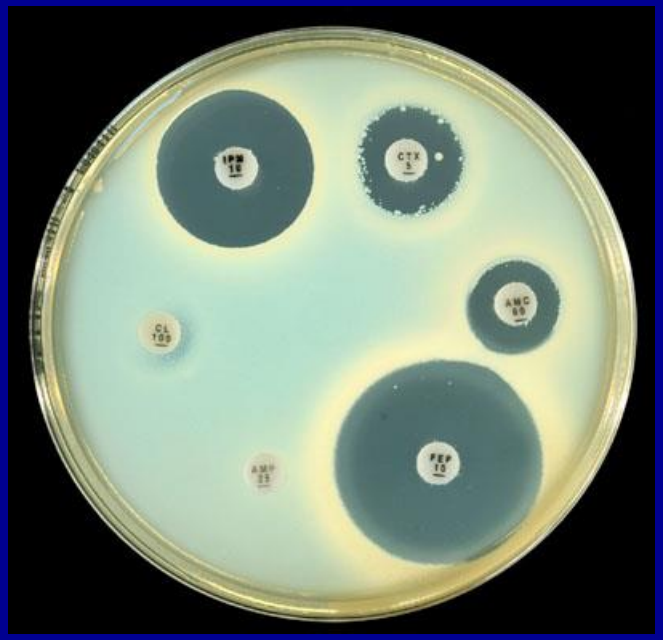
- Plasmid mediated and chromosomal

E.coli, K.pneumo
Salmonella sp

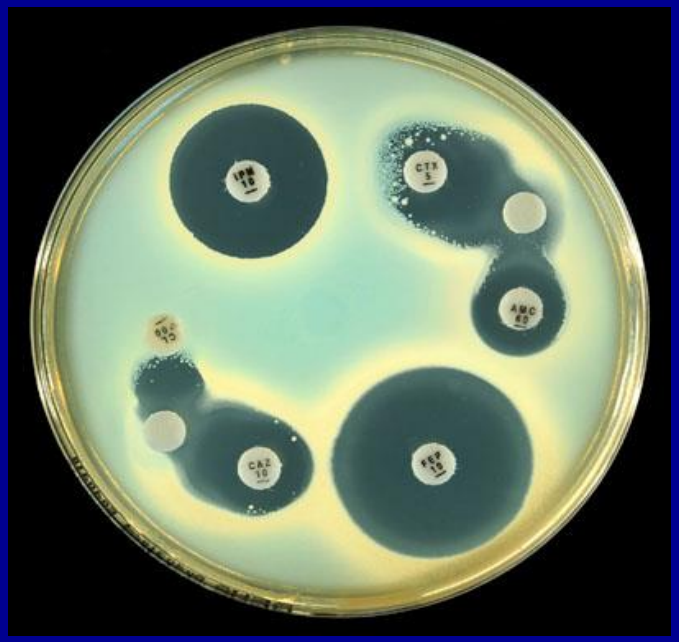
EEC group, S.marcescens,
H.alvei, P.stuartii,
P.rettgeri, M.morganii

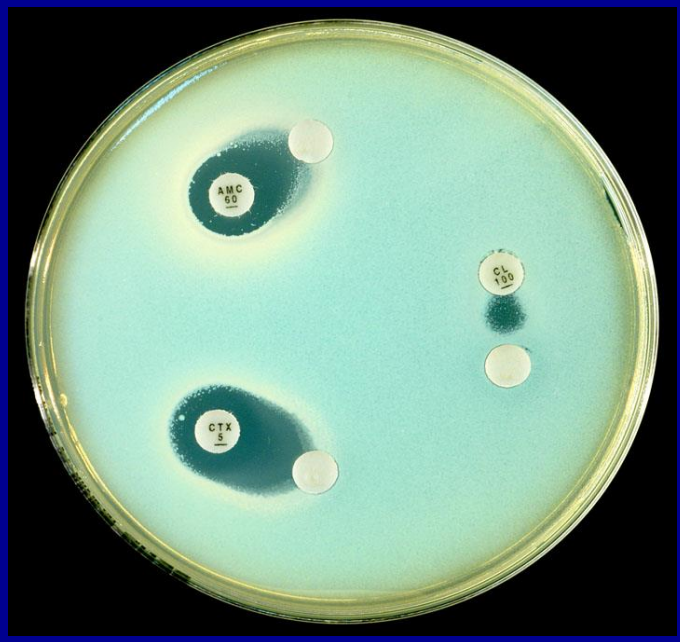
Plasmid Mediated AmpC in Routine CDS Testing

- R/ cephalexin 100ug, augmentin 60ug
- S/ cefepime (4th generation)
- S/ aztreonam
- Confirmation using boronic acid



Confirmation of AmpC with Boronic Acid

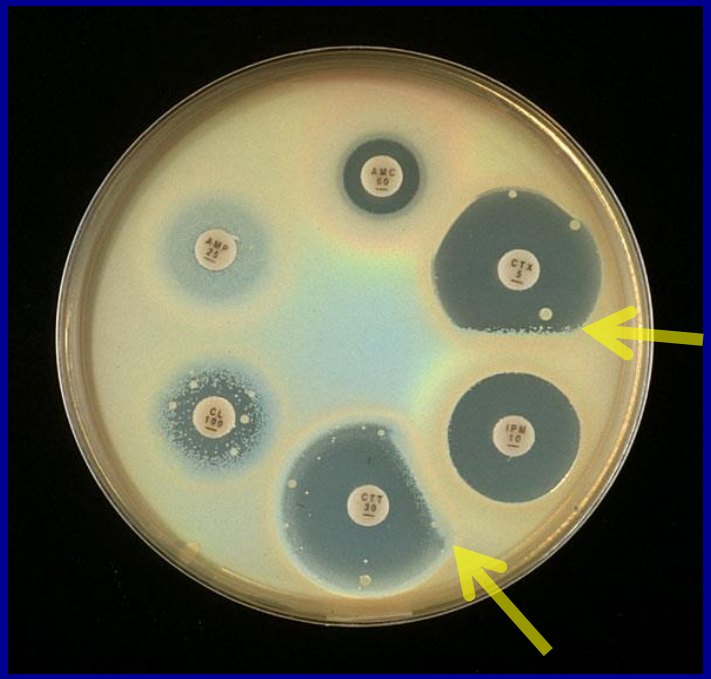




Same *E.coli* showing synergy between boronic acid discs and adjacent cefotaxime, Augmentin, cephalexin and ceftazidime

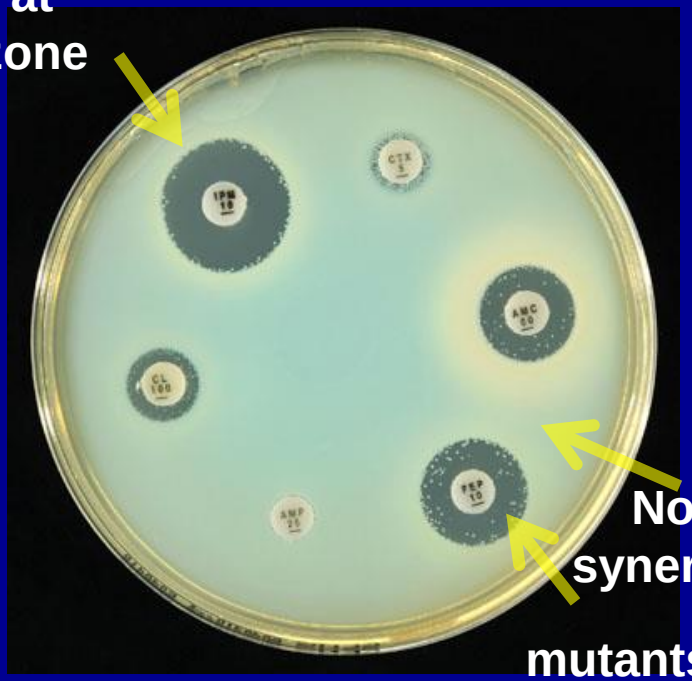
Chromosomal Inducible AmpC

- Four distinct sub groups
- Flattened inhibitory zone
- High mutation rate
- R/ CL, CTX, AMC
- S/ ATM



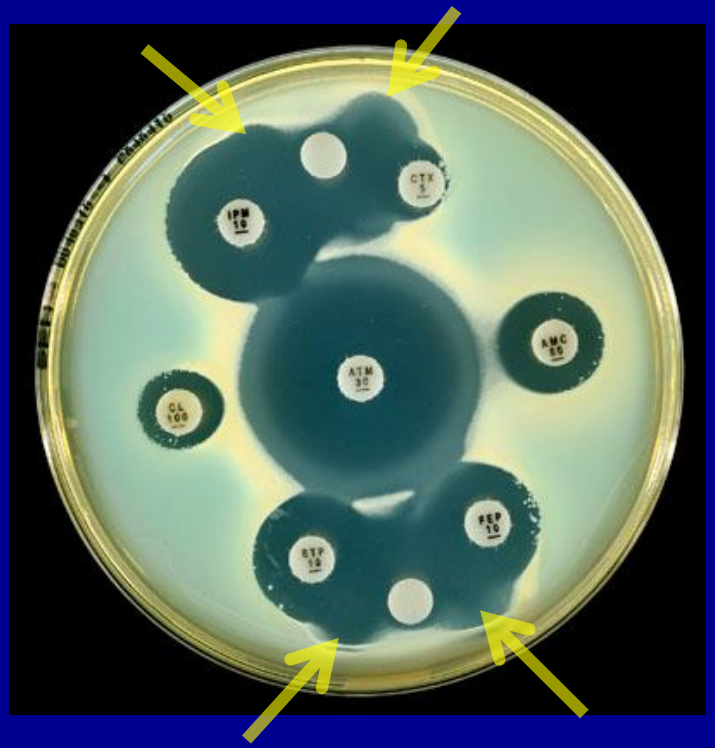
Metallo β-lactamase Characteristics

- Plasmid mediated β-lactamases
- R/ Penicillins, Cephalosporins, Augmentin, Carbapenems
- S/ Aztreonam
- Imipenem may be >6mm

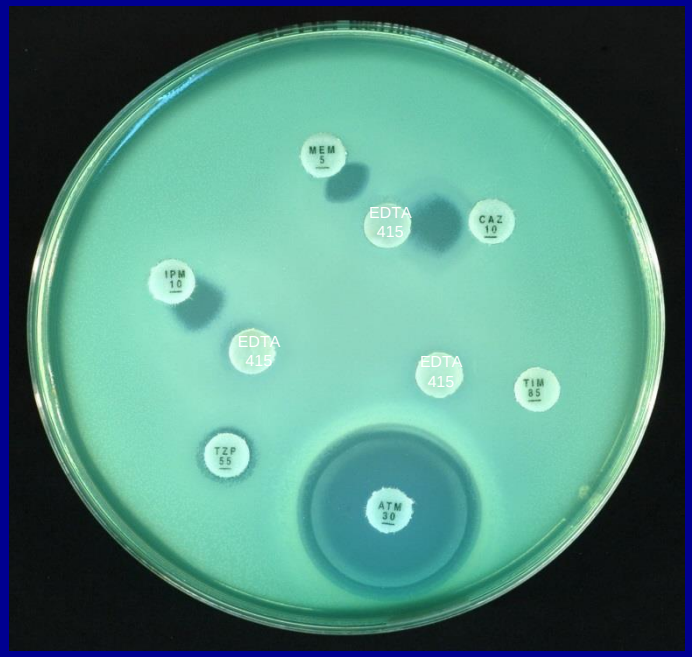


Confirmation Of MBL

- S/ Aztreonam
- Require zinc ions (EDTA disc test)
- Molecular detection



Pseudomonas aeruginosa and EDTA Testing

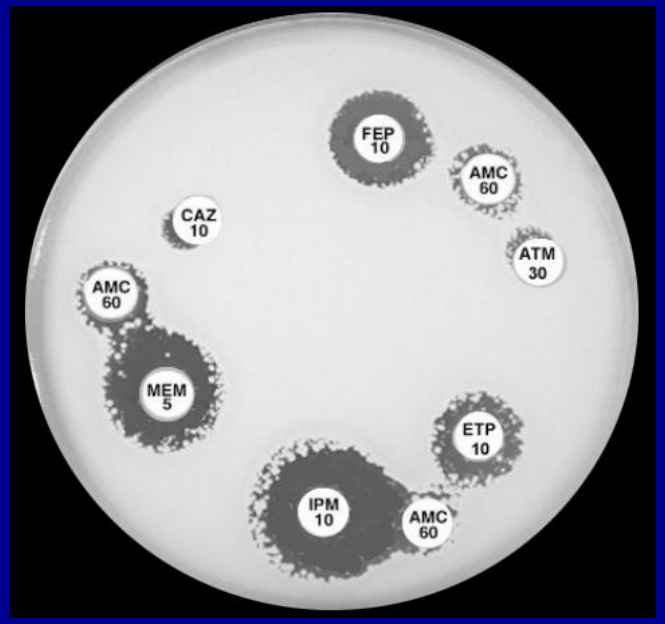


P.aeruginosa **showing synergy between EDTA and impenem, meropenem, ceftazidime**

Non-specific Synergy

Detecting Klebsiella pneumoniae Carbapenemases (KPCs) In Routine Testing

KPC in routine CDS test



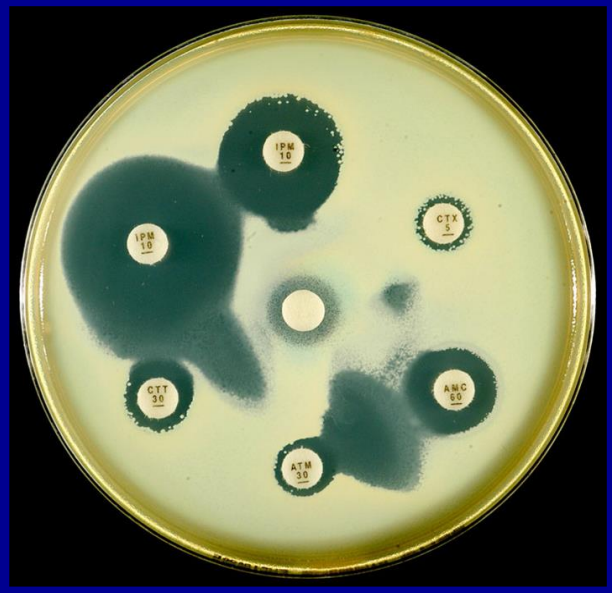
Confirmatory test: synergy between AMC 60/IPM 10

Oxa-48 and Oxa-181

- Oxa-48 and Oxa-181
- Presumptive ID by phenotypic method
- Detection by PCR

Co-expression Of Resistance

E.coli with PM-AmpC and ESBL



K.pneumoniae with MBL and ESBL

Summary

- CDS method can readily demonstrate and confirm a number of β-lactamase mediated resistance
- Access to hard copy manual, website and Reference Laboratory
- Detection of emerging resistance

Acknowledgements

- Special thanks to Professor Bell and Dianne Rafferty for their guidance and support
- SEALS Kogarah, especially A/Prof Peter Taylor and Chinmoy Mukerjee

Streptococcus spp. & *Haemophilus* spp. with decreased beta-lactam susceptibility: CDS reporting

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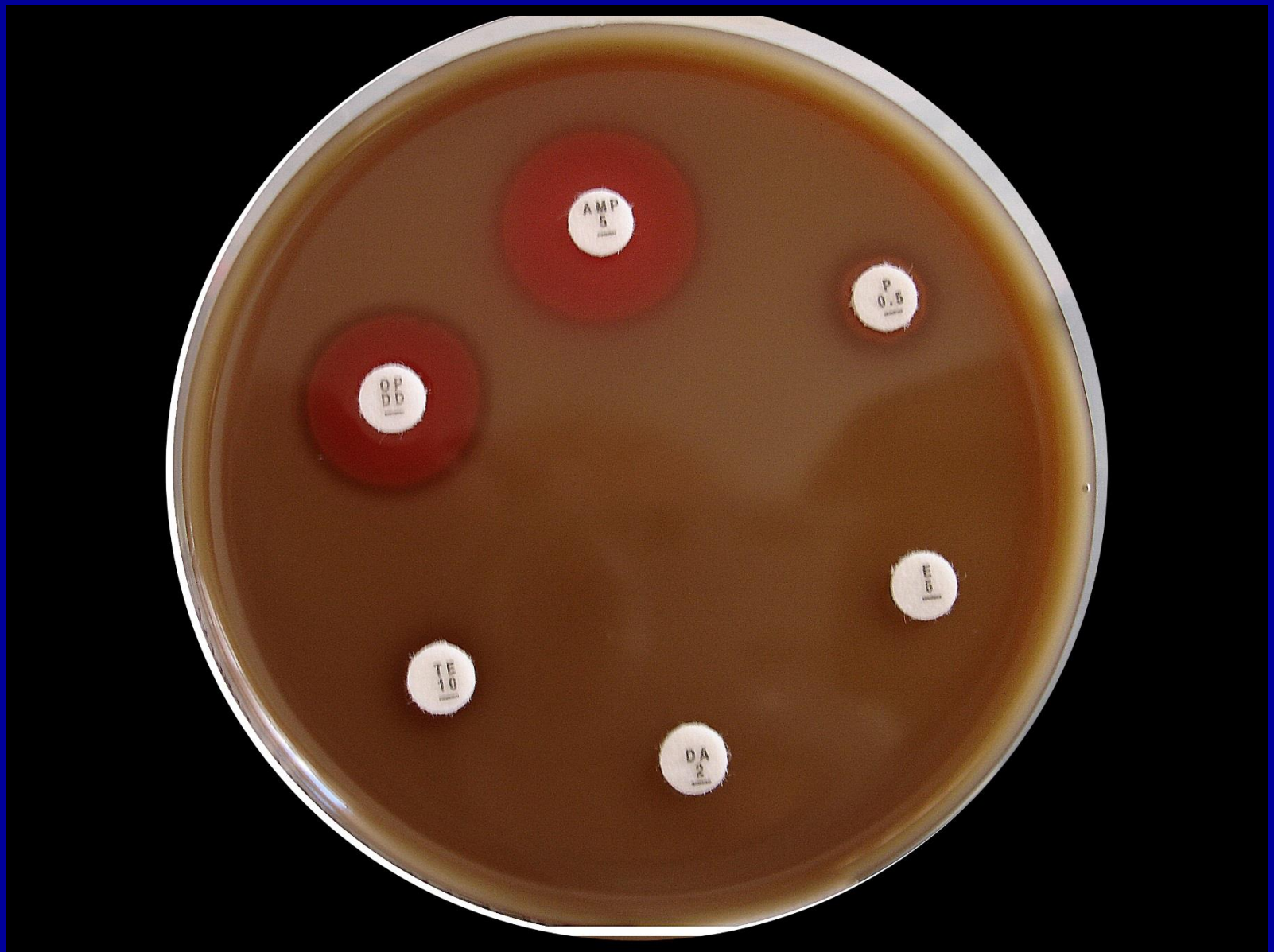
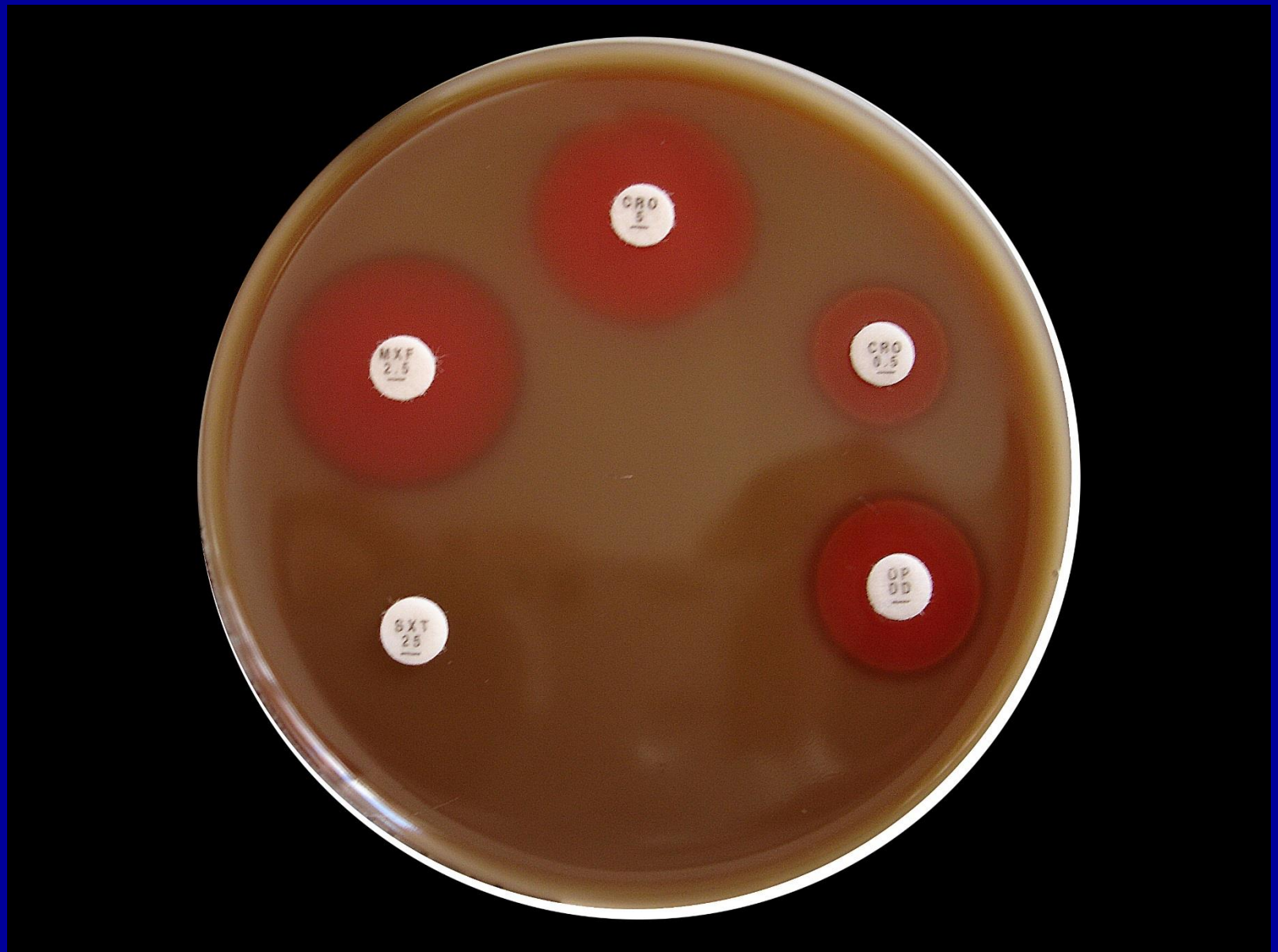
RCPA QAP 2015:8:3
Strep. pneumoniae from eye swab 10 y.o. child



RCPA QAP 2015:8:3
Strep. pneumoniae from eye swab 10 y.o. child



Streptococcus pneumoniae CDS reporting

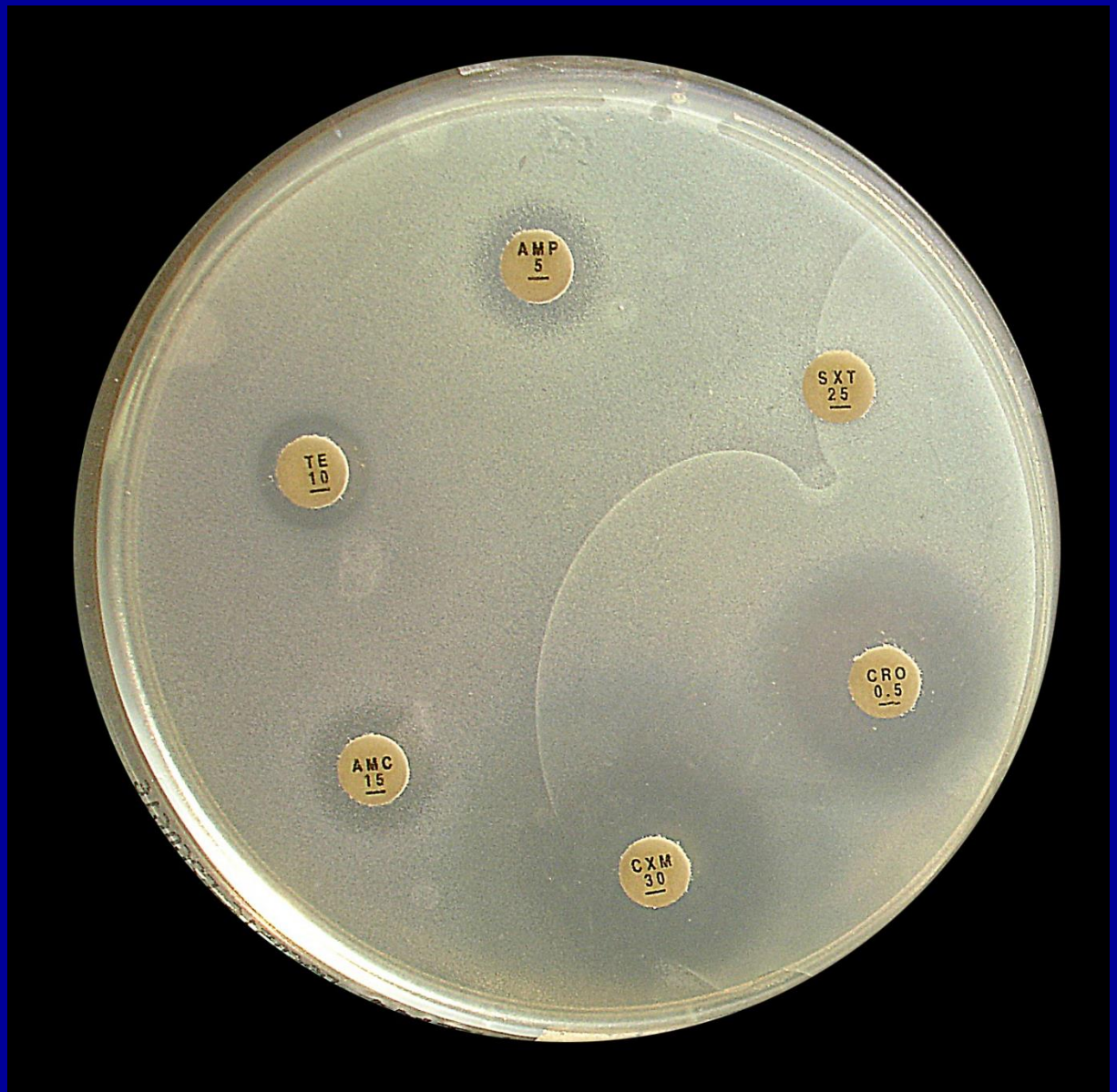
- From sites other than CSF (sputum, ear, eye & blood cultures not a/w meningitis)
- AR Pen 0.5u <6mm, Amp 5 µg ≥4mm: report DS for
  - Penicillin
  - Ampicillin
  - Amoxycillin
- Add comment: DS=There is decreased susceptibility to penicillin, ampicillin & amoxycillin with the MIC between 0.25 mg/L & 2.0 mg/L.
- Applies also to streptococci other than βHS groups A, C, G (e.g. GBS, viridans group, *S. milleri* group)
- If endocarditis perform M.I.C. determination

Streptococcus pneumoniae CDS reporting

- From sites other than CSF (sputum, ear, eye & blood cultures not a/w meningitis)
- AR CRO/CTX 0.5 µg <4mm, CRO/CTX 5 µg ≥6mm: report DS for
  - Cefotaxime
  - Ceftriaxone
- Comment: DS= There is decreased susceptibility to cefotaxime (or ceftriaxone) with the MIC between 1.0 mg/L and 2.0 mg/L.
- Applies also to streptococci other than βHS groups A, C, G (e.g. GBS, viridans group, *S. milleri* group)
- If endocarditis perform M.I.C. determination

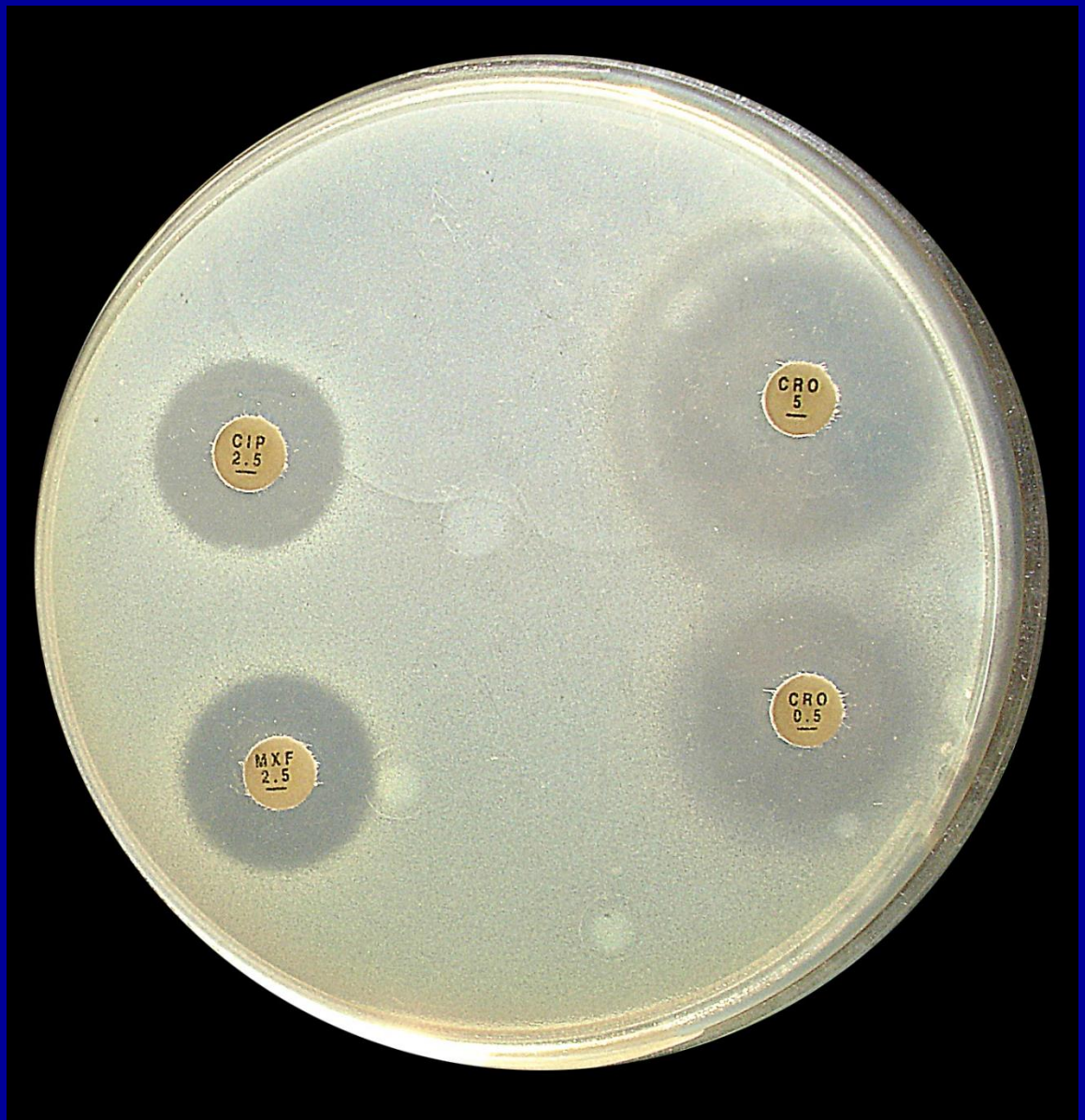
RCPA QAP 2015:7:2A

H. influenzae sputum isolate from a 48 y.o. female



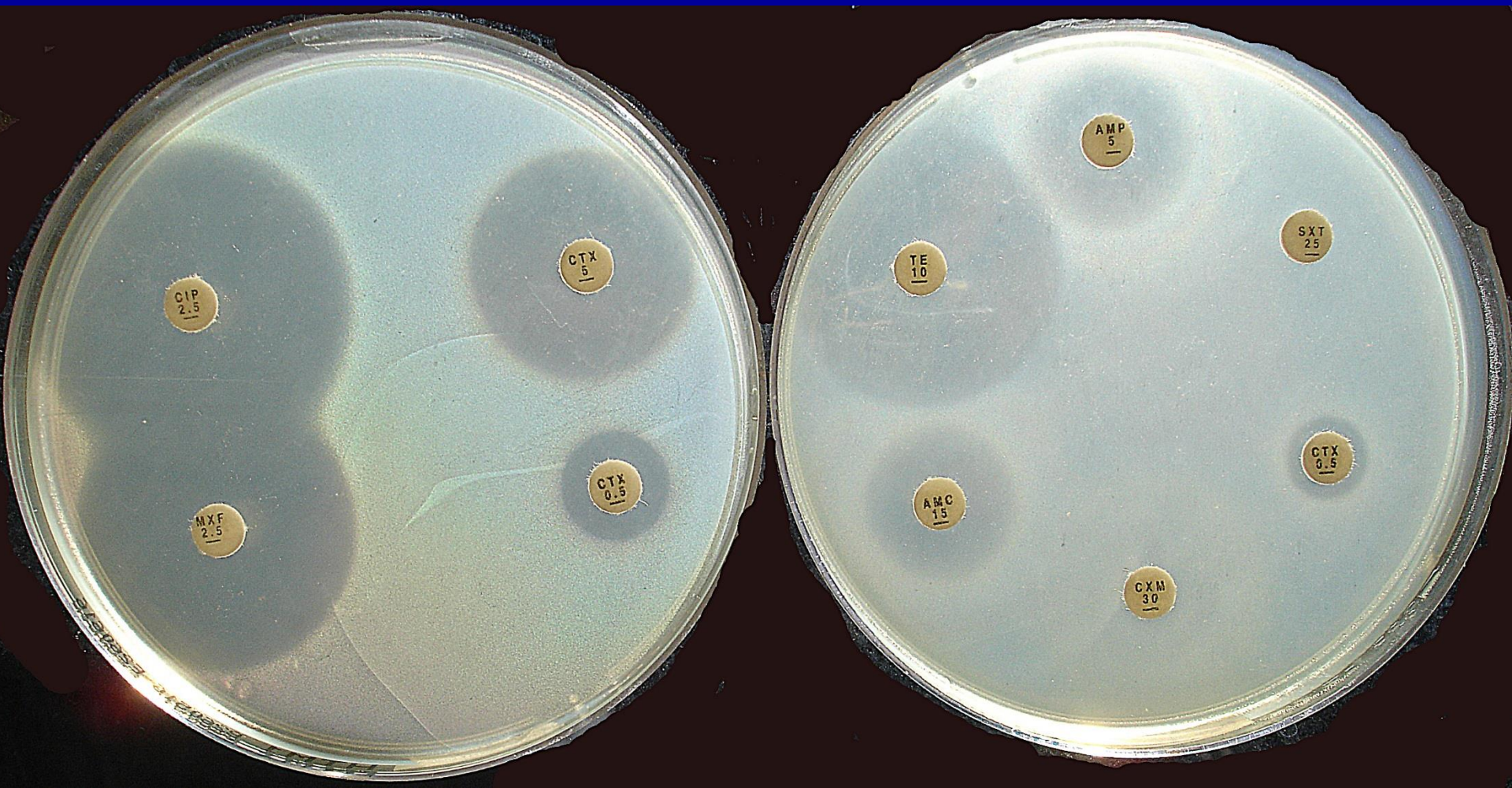
RCPA QAP 2015:7:2A

H. influenzae sputum isolate from a 48 y.o. female



A recent *H. influenzae* isolate from an 81 y.o. male with an infective exacerbation of COPD

β-lactamase negative



Beta-lactam resistance in *H. influenzae*

- β-lactamase mediated
  - Confers resistance to Ampicillin & Amoxycillin
  - TEM-1 (& less commonly ROB-1) β-lactamases
  - Inhibited by clavulanic acid (Augmentin S)
- Altered penicillin binding proteins (PBPs)
  - Lower affinity for β-lactams
  - β-lactamse-negative ampicillin-resistant (BLNAR) strains
  - Mutations in *ftsI* gene that encodes PBP3
  - Decreased susceptibility to ampicillin, amoxycillin, Augmentin & cephalosporins
- Rarely both mechanisms: (BLPACR strains)
  - β-lactamase-producing amoxycillin-clavulanate-resistant

CDS reporting of *Haemophilus* spp. with decreased susceptibility to Cefotaxime & Ceftriaxone

- AR CRO/CTX 0.5 µg <6mm, CRO/CTX 5 µg ≥6mm: report DS for
  - Cefotaxime
  - Ceftriaxone
- Add comment: DS= There is decreased susceptibility to cefotaxime (or ceftriaxone) with the MIC between 0.5 mg/L and 2.0 mg/L.

Recognition of OXA-48-like CPE with the CDS method: a case study

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Peter Newton

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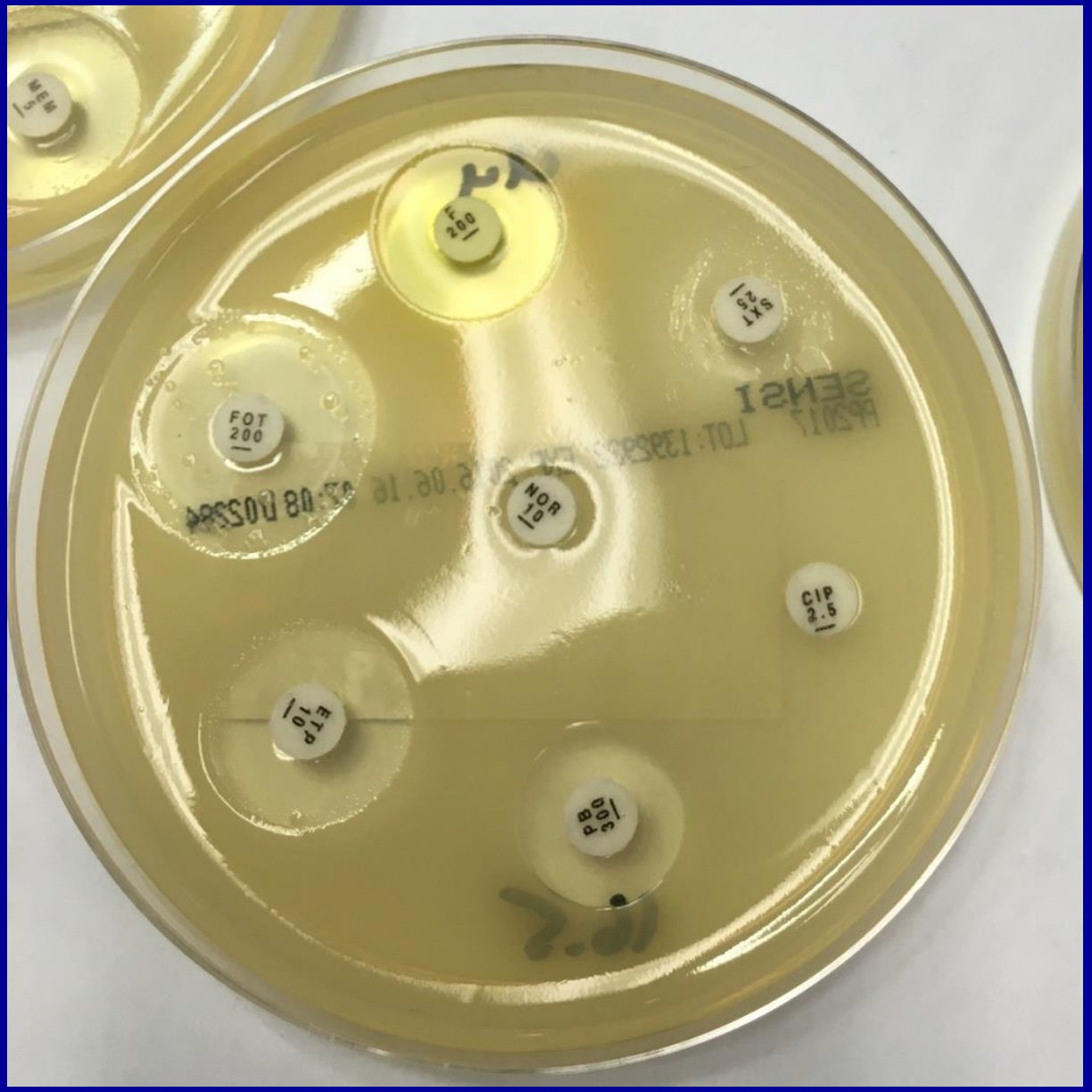
Case History

- 71 y.o. male Turkish immigrant
- Background:
  1. Metastatic colorectal cancer
     - Colostomy
     - Lung metastases on chemotherapy
  2. COPD
- Admitted with pulmonary embolus
  - Anticoagulated
- Day 10: retroperitoneal haemorrhage → hypovolaemic shock → ICU 2 days
- Day 13: *E. coli* UTI post removal of IDUC

AMP 25
FEP 10
IPM 10
TZP 85
CRO
AMC 60
CL 100

TOB 10
CN 10
ATM 30
AMC 60
W 5
K 30
MEM 10


FOT 200
NOR 10
SXT 25
CIP 2.5
ETP 10
PB 300

Further testing

- CarbaNP test positive

- No MBL detected using EDTA disc
- CARAlert referral made
- Multiplex PCR for resistance determinants:
  - blaOXA-48-like gene detected

Vitek 2 AST-N246 result

Identification Information	
Selected Organism	Escherichia coli
	Entered: May 19, 2016 16:13 EST By: labtech

álysis Messages:

The following antibiotic(s) are suppressed from analysis:
Ticarcillin/Clavulanic Acid,

Susceptibility Information	Card: AST-N246		Lot Number: 646386210	Expires: Jul 11, 2017 11:00 EST	
	Completed: May 20, 2016 00:15 EST		Status: Final	Analysis Time: 8.00 hours	
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ampicillin	>= 32	R	Meropenem	<= 0.25	S
Amoxicillin/Clavulanic Acid	>= 32	R	Amikacin	<= 2	S
Ticarcillin/Clavulanic Acid			Gentamicin	<= 1	S
Piperacillin/Tazobactam	>= 128	R	Tobramycin	2	S
Cefazolin	32	R	Ciprofloxacin	>= 4	R
Cefoxitin	<= 4	S	Norfloxacin	>= 16	R
eftazidime	<= 1	S	Nitrofurantoin	<= 16	S
Ceftriaxone	<= 1	S	Trimethoprim	<= 0.5	S
Cefepime	<= 1	S	Trimethoprim/Sulfamethoxazole	>= 320	R

+= Deduced drug *= AES modified **= User modified

AES Findings:	Last Modified: Sep 1, 2014 12:25 EST	Parameter Set: SESIAHS Copy of Global+Natural Resistance
Confidence Level:	Inconsistent	

CPE: Carbapenemase-producing *Enterobacteriaceae*

- Carbapenemases in 3 Ambler classes
- Class A: mainly KPCs
  - Inhibited by clavulanic acid & tazobactam
- Class B: metallo-β-lactamases (MBLs)
  - IMP, VIM, NDM etc.
  - Inhibited by chelators (EDTA & dipicolinic acid)
  - Most hydrolyse Aztreonam poorly
- Class D: oxacillinases, mainly OXA-48-like
  - Weakly hydrolyse carbapenems
  - Do not hydrolyse cephalosporins

OXA-48-like carbapenemases

- OXA-48 enzyme first identified in a *K. pneumoniae* isolate in Turkey
- Now in *Enterobacteriaceae* in USA, Europe, the Middle East, and Northern Africa
- Relatively rare in Australia (<=NDM & KPC<<<IMP; Vic > NSW) (CARAlert May 2016 Report)
- Weakly hydrolyse carbapenems
  - Can test carbapenem S
  - High level carbapenem R if a/w porin changes
- Usually test cephalosporin S
  - Unless isolate also carries an ESBL or AmpC

Clues to OXA-48 with CDS

- Imipenem annular radius (AR)<6mm or 6-7 mm with mutant colonies at zone edge

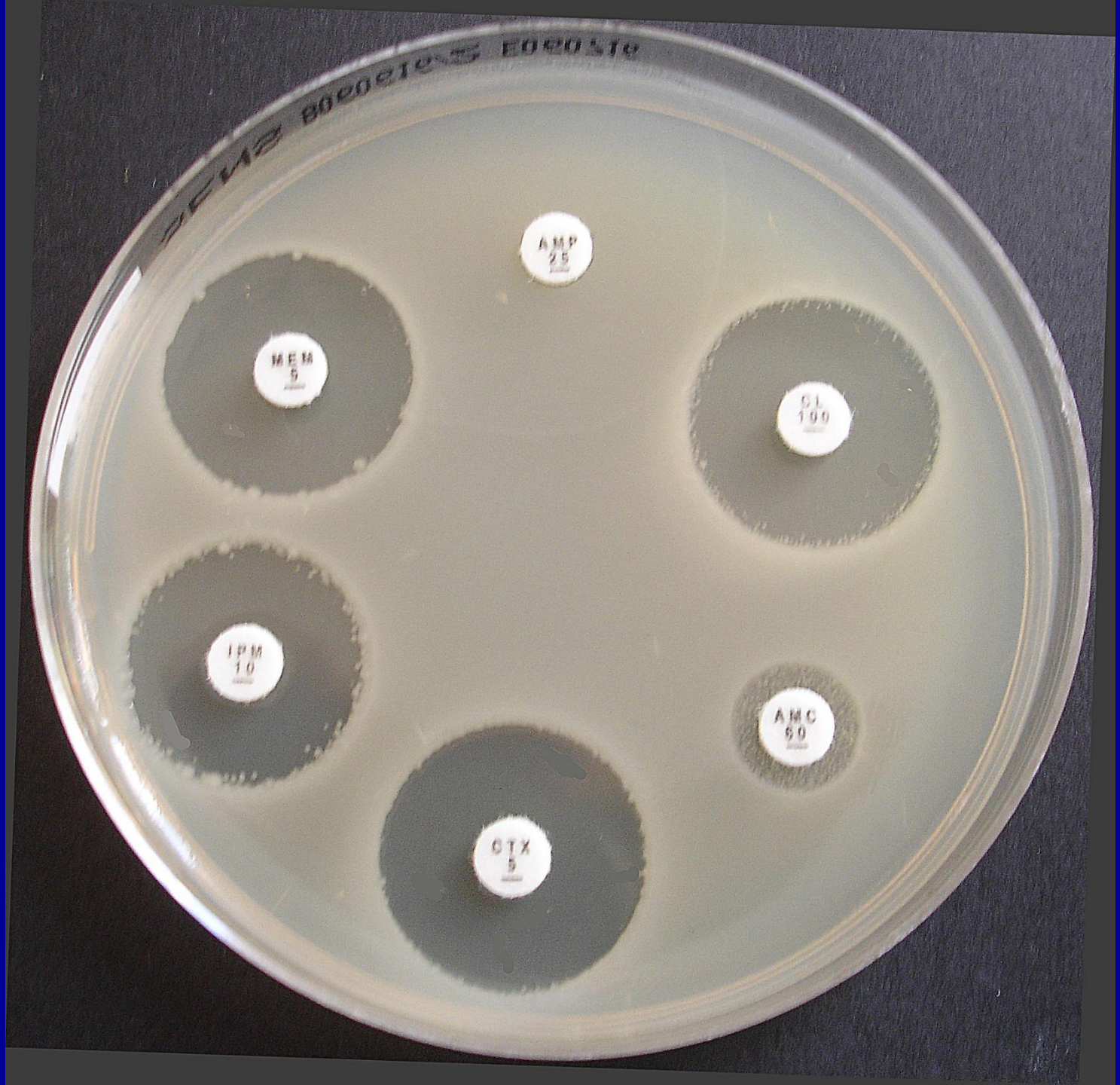
  Plus

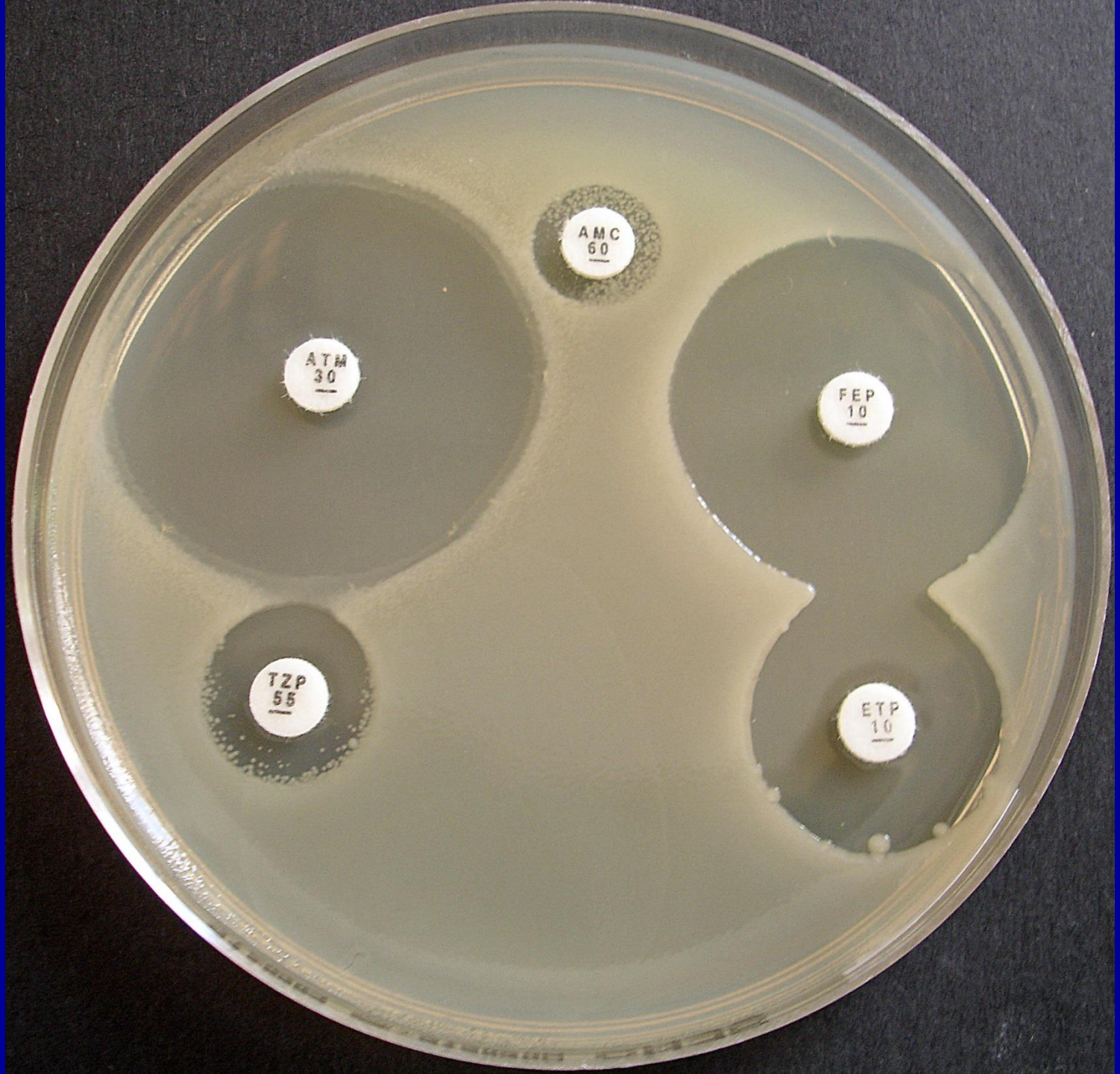
- Resistant to Augmentin & Tazocin

  Plus

- Cefotaxime/Ceftriaxone & Cefepime AR > carbapenems

- Perform further confirmatory tests or send to CDS lab


AMP 25
MEM 5
CL 100
IPM 10
AMC 60
CTX 5


AMC
60
ATM
30
FEP
10
TZP
55
ETP
10