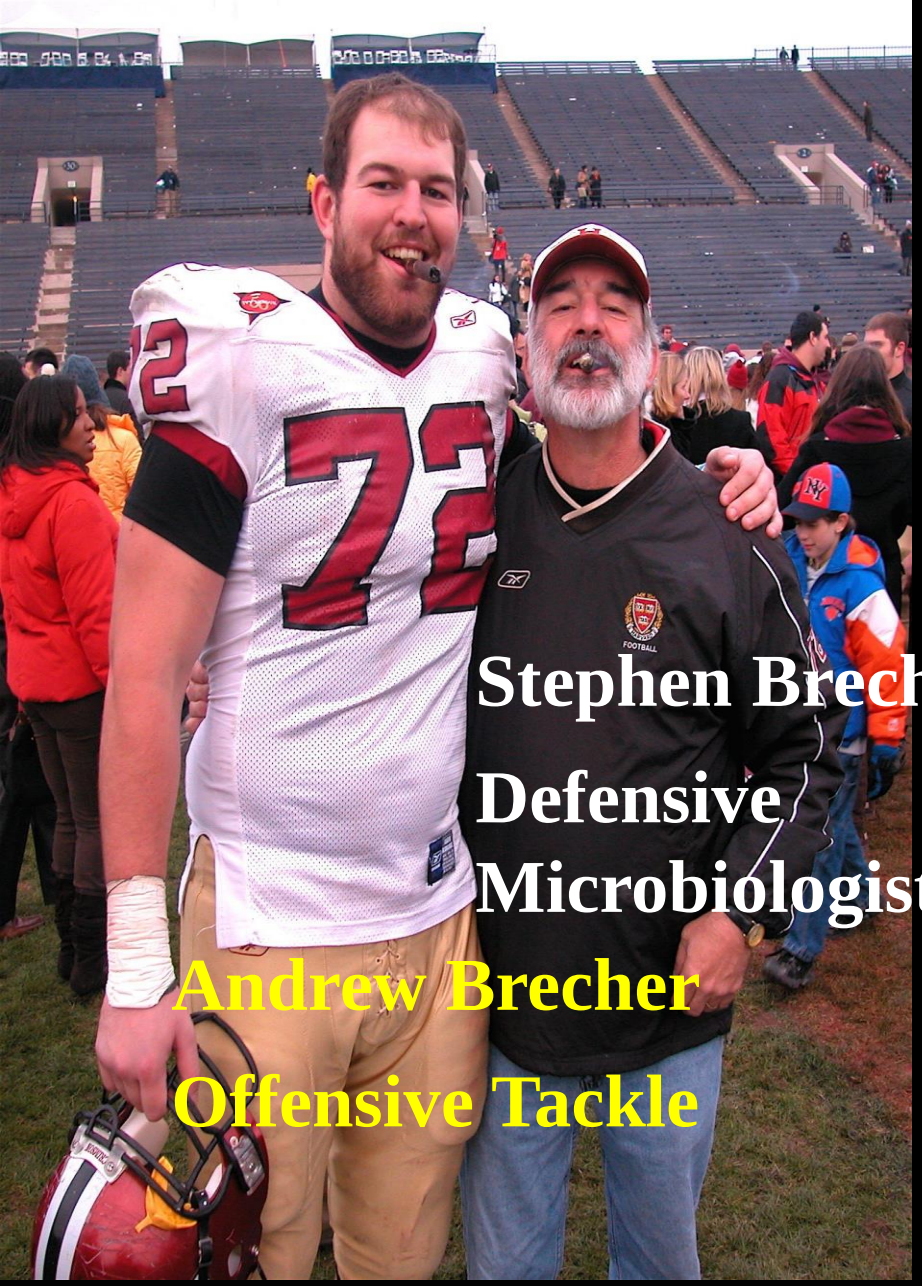
Tackling MDROs in the Clinical Laboratory



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VA Boston Healthcare System
Associate Professor of Pathology
and Laboratory Medicine
BU School of Medicine

Overview

- Case Report
- Enterobacteriaceae
  - CLSI changes
  - Definitions of beta-lactamases, ESBLs, ampCs, and CREs
  - Laboratory detection issues
- Staphylococci
  - Testing issues
  - mecC
  - Vancomycin
- Lunch Recommendation


We do not have resistant bacteria or nosocomial infections in our hospital

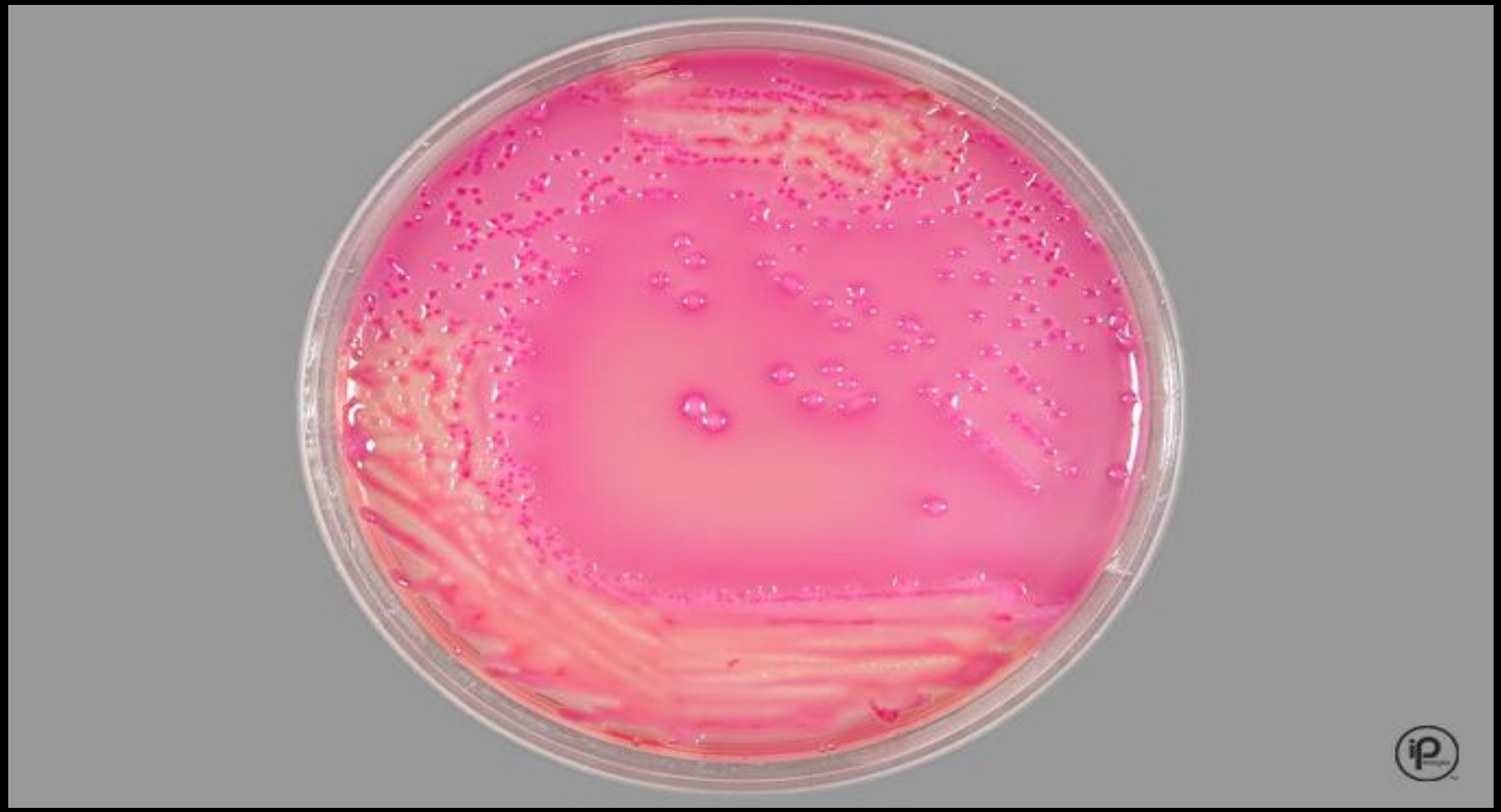
Case Study
(slides from Dr. Stephen Jenkins)

- A 48 year old obese female was admitted for elective knee replacement surgery following an automobile accident
- Post-surgery she had idiopathic heparin-induced thrombocytopenia
- Loss of perfusion to her intestines resulted in small bowel transplant
- Post-surgery day 2 she developed ARDS and was placed on a ventilator
- The patient's condition continued to deteriorate and she developed a nosocomial pneumonia

Case Study

A gram-negative enteric like organism was recovered from BAL, an empyema collection, urine, and blood

Klebsiella pneumoniae (KPC/CRE)



Antibiotic Susceptibility Testing

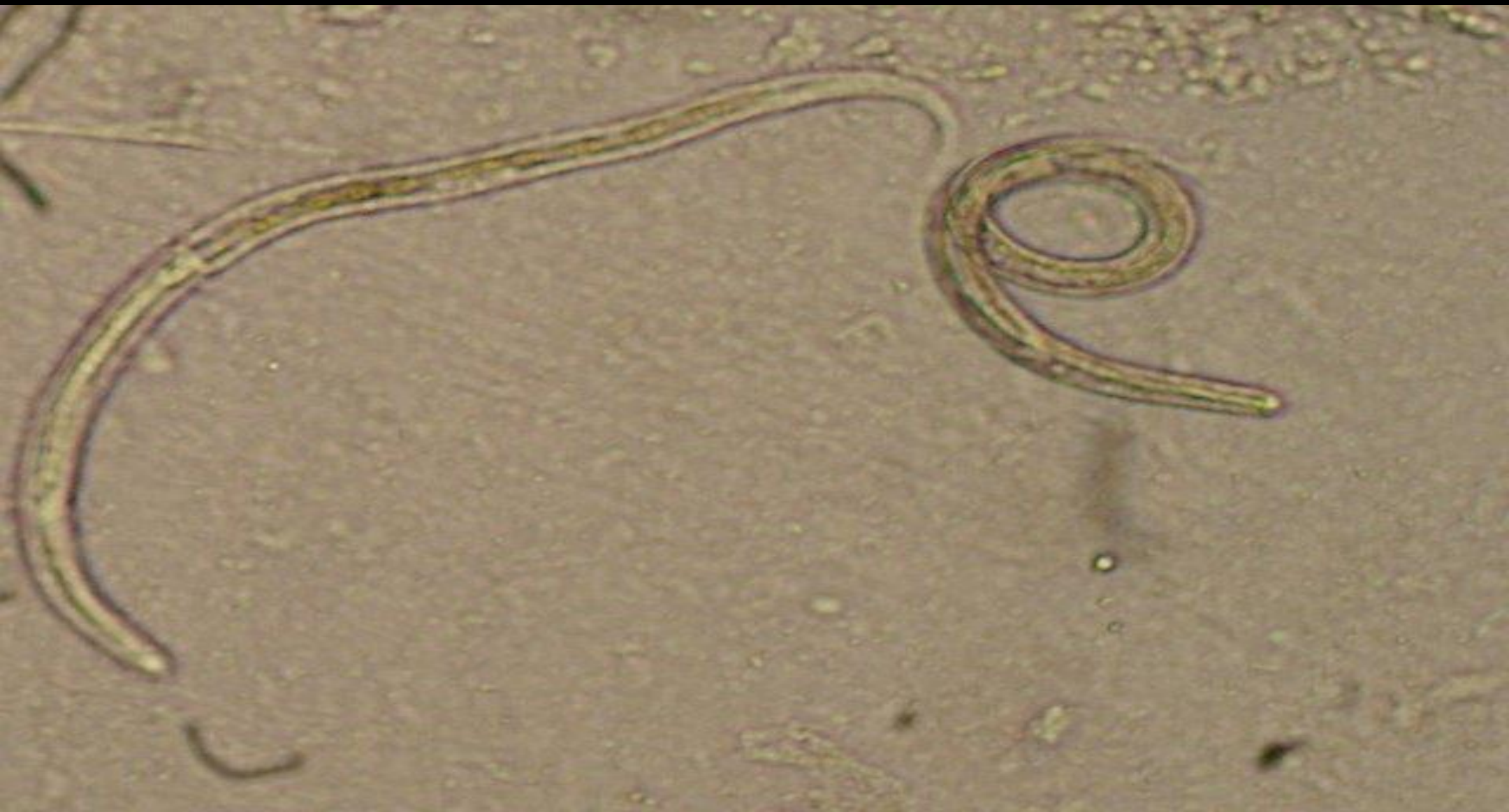
Subsequent Stool Isolate

Isolate	***Klebsiella pneumoniae***	
ANTIBIOTICS	MIC (µg/mL)	
Ampicillin	>16	R
Aztreonam	>16	R
Ceftriaxone	>32	R
Ceftazidime	>16	R
Cefotaxime	>32	R
Cefazolin	>16	R
Ciprofloxacin	>2	R
Cefepime	>16	R
Cefuroxime	>16	R
Amikacin	**32**	**I**
Imipenem	>8	R
Meropenem	>8	R
Ertapenem	>4	R
Polymyxin B	**2**	**S (?)**
Gentamicin	8	R
Levofloxacin	>4	R
Meropenem	>8	R
Trim/Sulf	>2/38	R
Tetracycline	>8	R
Tobramycin	>8	R

Treatment of CRE

- Polymyxin B MIC = 2 μg/mL (Susceptible?)
- Patient treated with tigecycline and polymyxin B - responded
- Reports in the literature of successful treatment of this organism with polymyxin B plus rifampin and combinations of agents that include imipenem and/or an aminoglycoside

The Patient Developed a Second Pneumonia Related to:

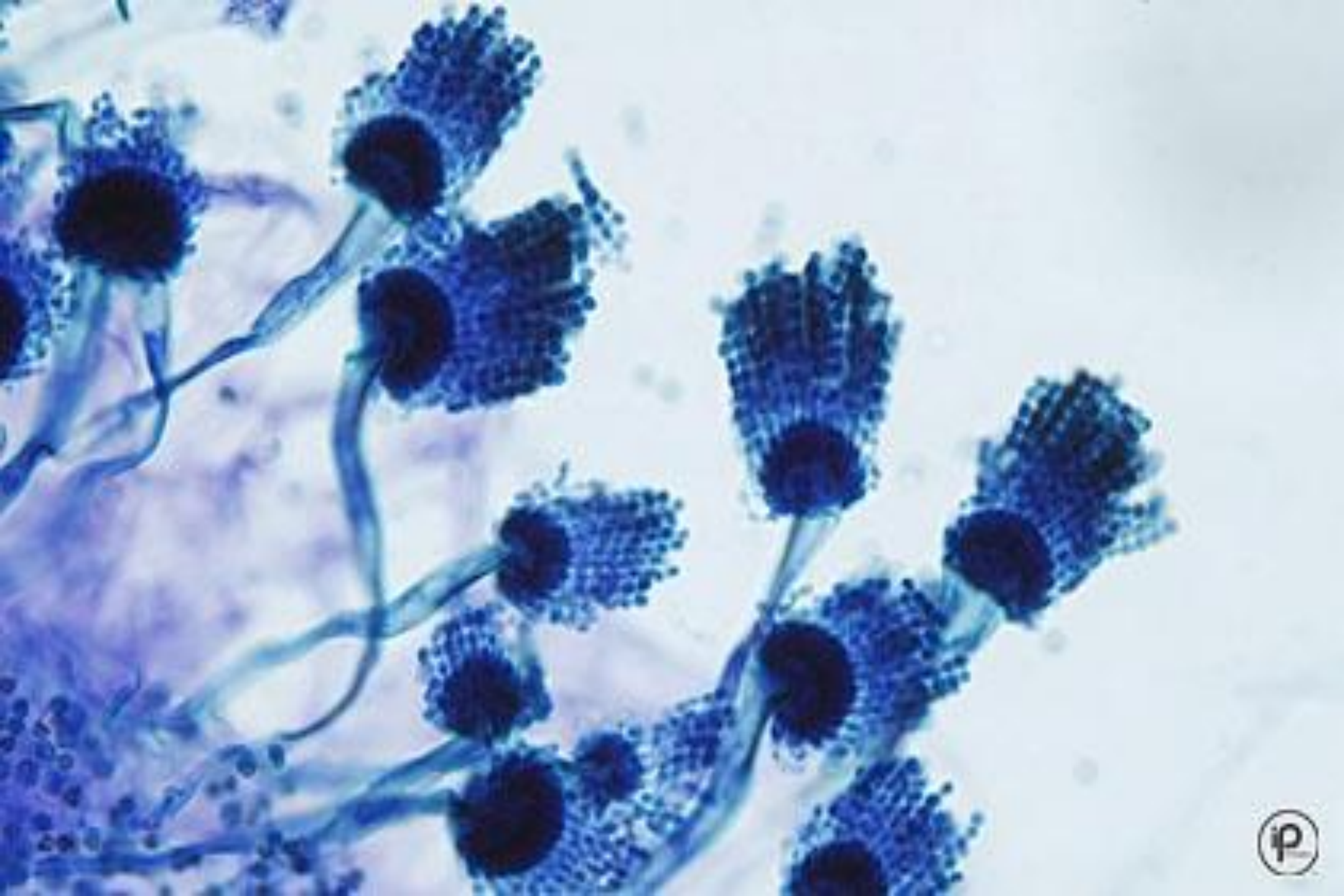


Follow-up

Hyperinfestation with *Strongyloides stercoralis*

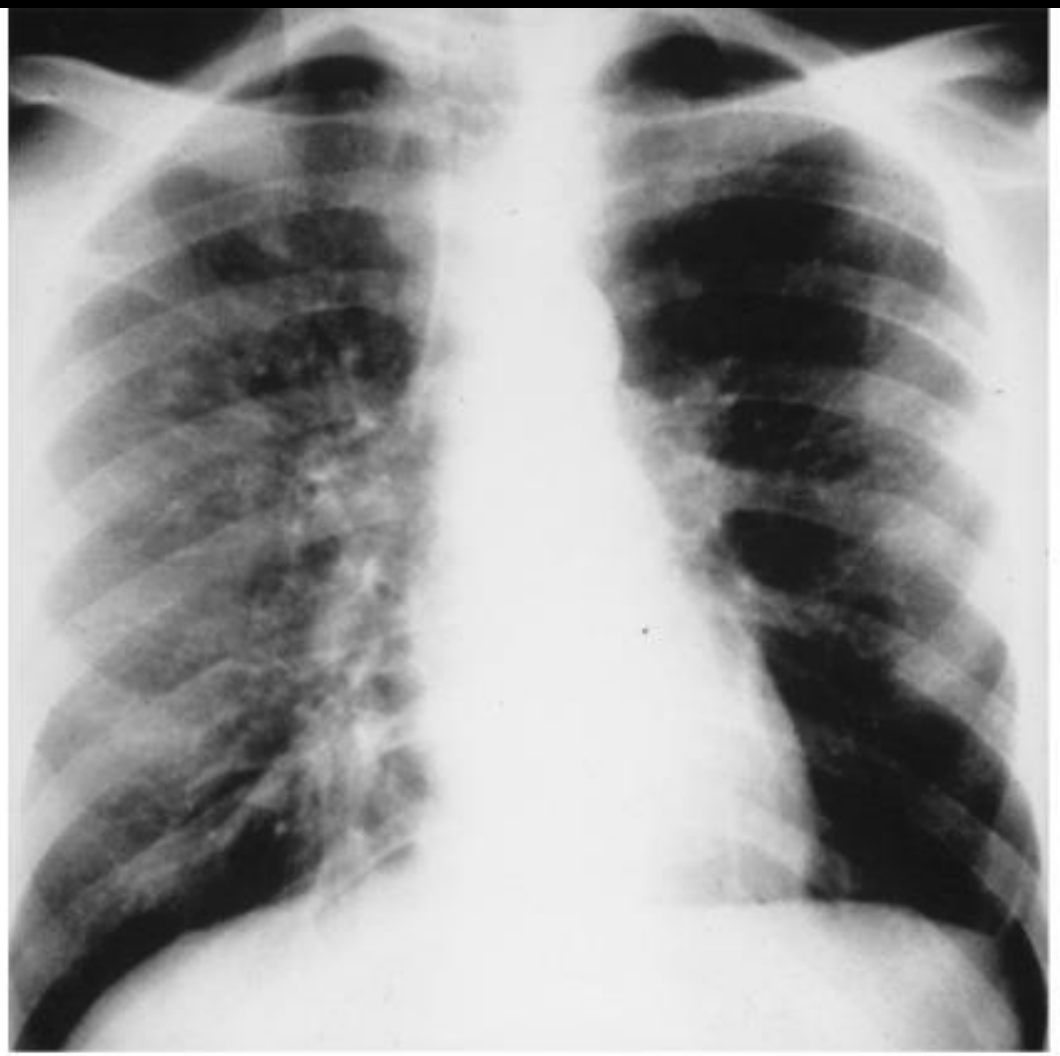
Follow-up

Treated and recovered, only to develop a new pneumonia with:



Follow-up

- ***Aspergillus fumigatus***
- **Again responded to therapy (voriconazole), but developed bilateral CMV pneumonia**



Follow-up

Controlled with high-dose gancyclovir, but became septic with:

Multi-drug resistant strain of *Acinetobacter baumannii*

- β-lactam (including imipenem), aminoglycoside, and fluoroquinolone resistant
- Expired 13 months after initial surgery

Why Do Bacteria Become Resistant to Antibiotics?

- We are trying to kill them
- They are trying to eat and reproduce
- What would you do if someone was trying to kill you while you were trying eat and/or reproduce?

CLSI Breakpoints Changes

Cephalosporin Changes

Enterobacteriaceae

Agent	CLSI 2009			CLSI after 2010		
	S	I	R	S	I	R
Cefazolin	≤ 8	16	≥ 32	≤ 2	4	≥ 8
Cefotaxime	≤ 8	16-32	≥ 64	≤ 1	2	≥ 4
Ceftizoxime	≤ 8	16-32	≥ 64	≤ 1	2	≥ 4
Ceftriaxone	≤ 8	16-32	≥ 64	≤ 1	2	≥ 4
Ceftazidime	≤ 8	16	≥ 32	≤ 4	8	≥ 16
Aztreonam	≤ 8	16	≥ 32	≤ 4	8	≥ 16

Cefepime 2014

Enterobacteriaceae

Agent	CLSI 2014		
	S	SDD	R
Cefepime	≤ 2	4-8	≥16

Susceptible Dose-Dependent (SDD) is based on dosing regimens that result in higher cefepime exposure

Cephalosporin/Cephamycin Non-Changes

Enterobacteriaceae

Agent	CLSI 2014		
	S	I	R
Cefuroxime	≤ 8	16	≥ 32
Cefotetan	≤ 16	32	≥ 64
Cefoxitin	≤ 8	16	≥ 32

Carbapenem Changes

Enterobacteriaceae

Agent	CLSI 2009			CLSI 2014		
	S	I	R	S	I	R
Doripenem	-	-	-	≤ 1	2	≥ 4
Ertapenem	≤ 2	4	≥ 8	≤ 0.5	1.0	≥ 2
Imipenem	≤ 4	8	≥ 16	≤ 1	2	≥ 4
Meropenem	≤ 4	8	≥ 16	≤ 1	2	≥ 4

A Primer on Beta-Lactamases

Classes of βeta-Lactamases

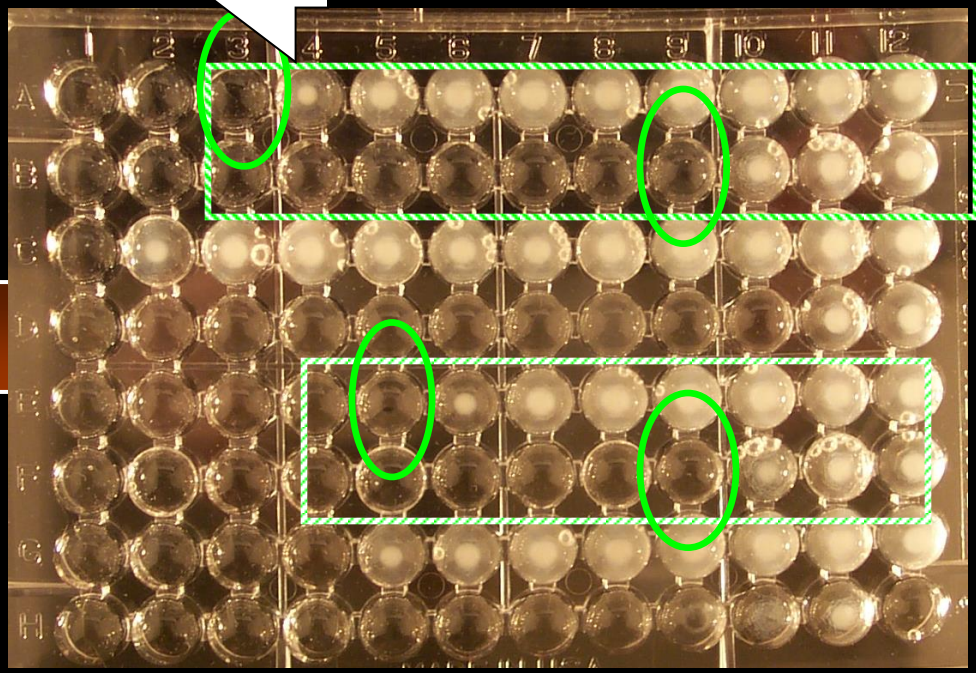
- **Molecular class A (TEM, SHV, ESBLs, CTX-M, KPCs)**
- **Molecular class B (metallo-β-lactamases: NDM, IMP, VIM, SPM)**
- **Molecular class C (AMP C: SPICE/SPACE bacteria)**
- **Molecular class D (OXA)**
- **There are > 1700 distinct β-lactamases**

Bradford PA. (2001) Clin Micro Rev; 14:933-951and Jacoby GA, Minoz-Price LS. (2005) NEJM; 352:380-391 for excellent reviews

Class A Extended Spectrum Beta-Lactamases (ESBLs)

- Bacterial enzymes produced primarily by *E. coli* and *Klebsiella species* that break down the beta-lactam ring of third and fourth generation cephalosporins
  - Ceftriaxone, cefotaxime, cefpodoxime, cetazidime, cefepime and aztreonam
  - Usually susceptible to the cephamycins (cefoxitin and cefotetan)
  - Inhibited by clavulanate, sulbactam, and tazobactam
  - Now seen in Salmonella, Proteus species and other enterics
  - Over 500 different ESBLs

CLSI ESBL MIC Confirmation Method: *K. pneumoniae* 700603



	MIC
Ceftazidime	32
Ceftazidime + Clav	0.5
Cefotaxime	8.0
Cefotaxime + Clav	0.5

Decrease in MIC by ≥3 dilutions with Clavulanic acid = ESBL

ESBL Disk Confirmation Test

If zone increases by > 5mm = ESBL

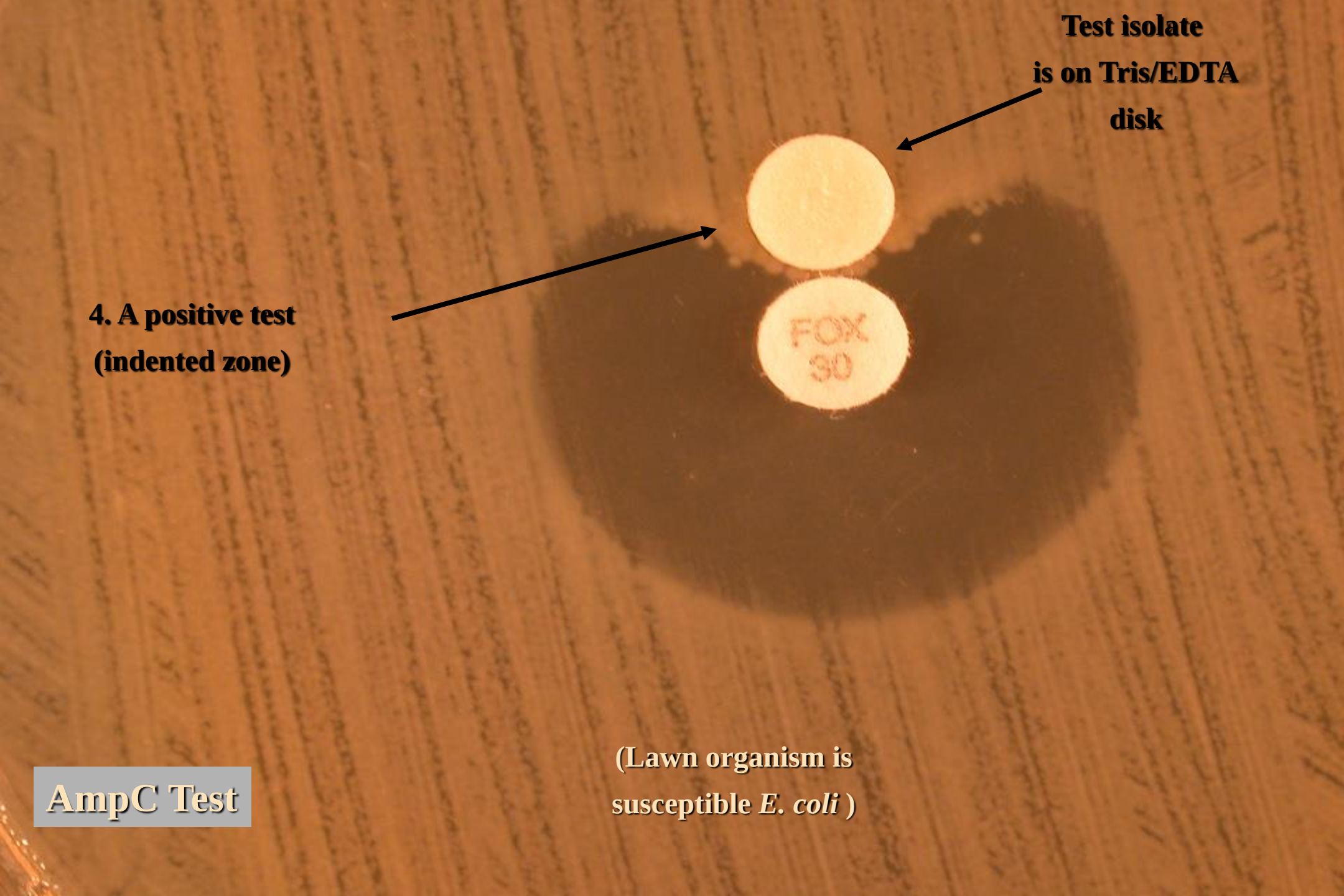
Do You Need to do This Test?

NO

(If you have changed to the current CLSI BPS)

ampC Beta-Lactamases (Class C)

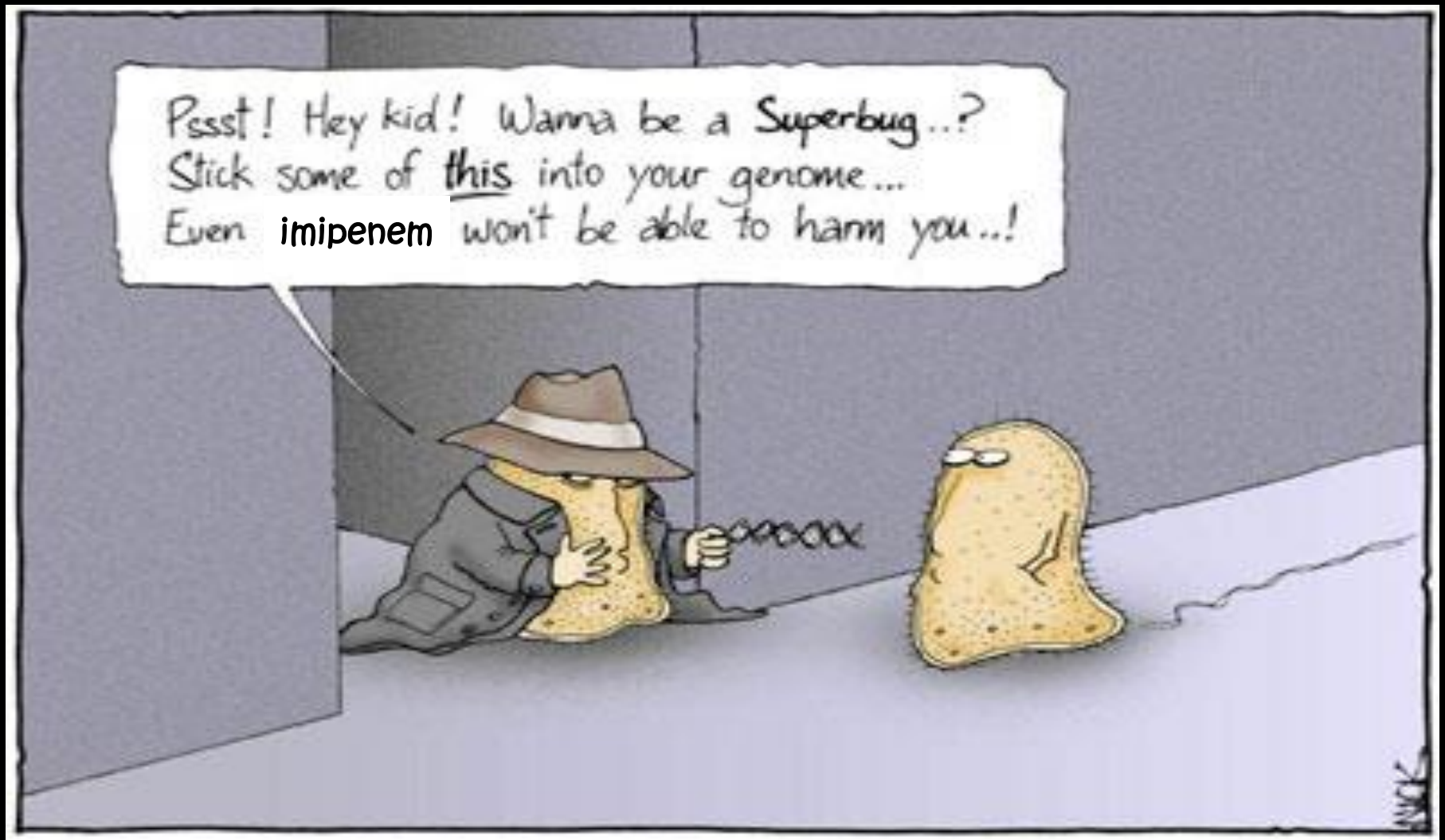
- **Found in Enterobacter, Serratia, and Citrobacter**
  - **Low level constitutive beta-lactamase production to inducible high-level beta-lactamase production**
    - **Selected during therapy**
  - **Induced by beta-lactam antibiotics**
  - **Not inhibited by clavulanate or tazobactam**
  - **Hydrolyze cephamycins and most cephalosporins, except cefepime**
  - **May hydrolyze carbapenems at very low rates**
  - **non-transferable (on chromosome)**
- **New: plasmid-mediated and transferable**
  - **Found in *E. coli, K. pneumoniae* and others**


Test isolate
is on Tris/EDTA
disk
4. A positive test
(indented zone)
FOX
30
(Lawn organism is
susceptible *E. coli*)
AmpC Test

Do You Need to do This Test?

NO

(If you have changed to the current CLSI BPS)



It was on a short-cut through the **Surgical and medical ICU** that Albert was first approached by a member of the Antibiotic Resistance.

Carbapenemases

- **Class A: KPC (24), SME, IMI, NMC**
  → serine residue at the active site
- **Class B: IMP (45), VIM (39), GIM, SPM, SIM, IND (15), NDM (16)**
  → Zn^{2+}- dependent metallo-enzyme
- **Class C: N/A**
- **Class D: OXA family (1 → 364), OXA 48**

Class A KPCs (CREs)

- ***Klebsiella pneumoniae* carbapenemase**
- **Mostly found in *K. pneumoniae*, but also in other enteric bacteria**
- ***KPC*$_{bla}$ resides in plasmids**
- **Hydrolyze all of the β-lactam antibiotics including cephalosporins and monobactams (as well as the carbapenems) → Very few therapeutic options**
- **24 distinct types identified so far (KPC 1, 2, etc.)**
- **Endemic in NYC; spreading across nation / world**

Class B Plasmid-Mediated Metallo-β-Lactamases

- **Zinc containing β-lactamases: not inhibited by clavulanic acid, tazobactam, or sulbactam**
- **Low rates of aztreonam hydrolysis**
- **Common in *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Enterobacteriaceae* (outside of US)**
- **Carbapenemase of *Stenotrophomonas maltophilia***

Class B Plasmid-Mediated Metallo-β-Lactamases

- **NDM-1: New Delhi metallo-β-lactamase**
- **First 3 *bla*$_{NDM-1}$ isolates detected in US were in *E. coli, Enterobacter cloacae, Klebsiella pneumoniae***
- **NDM-1 has quickly spread among non-clonally related isolates: *Citrobacter freundii, Morganella morganii, Providencia rettgeri, Acinetobacter baumannii, Providencia stuartii***
- **Confers resistance to all β-lactams except aztreonam**
- **Plasmid also carries other β-lactamases and genes conferring resistance to other classes of antibiotics (these 3 isolates were aztreonam-R due to other β-lactamases)**
- **Now have 16 different NDMs**

NDM-β-Lactamases

- Medical Tourism
  - "Clinicians should be aware of the possibility of NDM producing Enterobacteriaceae in patients who have received medical care in India and Pakistan and should specifically inquire about this risk factor when carbapenem-resistant enterics are reported"
- Isolates should be forwarded to CDC for confirmation (at least for now)

Class D Carbapenemases

- **Originally described as OXA Beta-lactamases that could hydrolyze oxacillin and cloxacillin, but they also hydrolyze carbapenems**
- **5 OXA Families**
  - **Multiple enzymes in each family**
- **Primarily found in Acinetobacter, Pseudomonas and Enterobacteriaceae**
- **Not influenced by inhibitors such as EDTA or clavulanic acid**
- **Mucoid *K. pneumoniae* with OXA-48 problematic in many parts of the world (but not NA)**

Laboratory Detection Of Carbapenem Resistance in Enterobacteriaceae

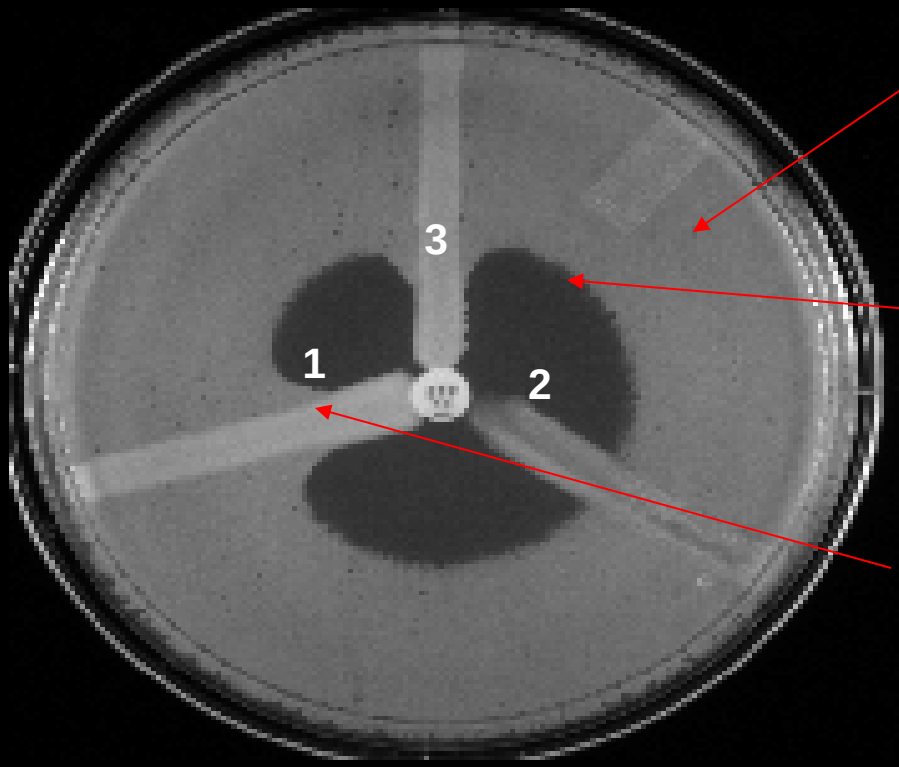

IPM
10
ETP

E-Tests for 4 Carbapenems

Non-Carbapenemase Carbapenem-Resistance

- Elevated carbapenem MICs are also associated with the following scenarios
  - An ESBL beta-lactamase and a porin protein mutation (permeability)
    - More common for ertapenem
  - An ampC beta-lactamase and a porin protein mutation
  - Imipenem resistance in Proteus, Providencia and Morganella (low level)

Modified Hodge Test
(Carbapenem Inactivation Test)



***E. coli* ATCC 25922**

Inhibition of *E. coli* ATCC 25922 by ertapenem

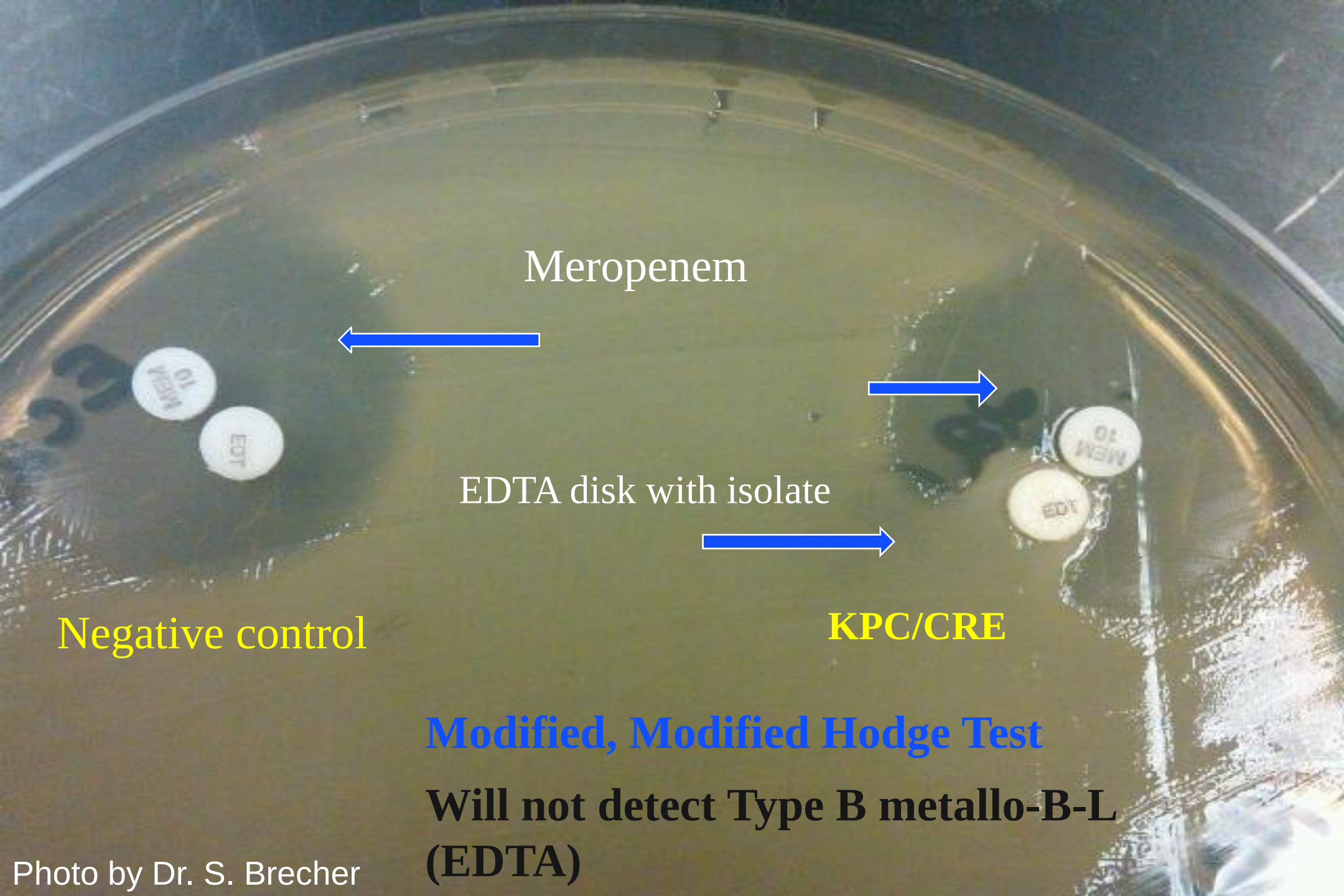
Enhanced growth of *E. coli* ATCC 25922. Carbapenemase produced by *K. pneumoniae* D-05 destroyed ertapenem that diffused into the media. Thus, there is no longer sufficient ertapenem to inhibit *E. coli* ATCC 25922 and an indentation of the zone is noted.

The MHT performed on a small MHA plate.

(1) *K. pneumoniae D-05*, positive result;

(2) *K. pneumoniae* 6179, negative result; and

(3) a clinical isolate, positive result



Modified, Modified Hodge Test

Will not detect Type B metallo-B-L (EDTA)

Photo by Dr. S. Brecher

Do You Need to do This Test?

NO

(If you have changed to the current CLSI BPS)

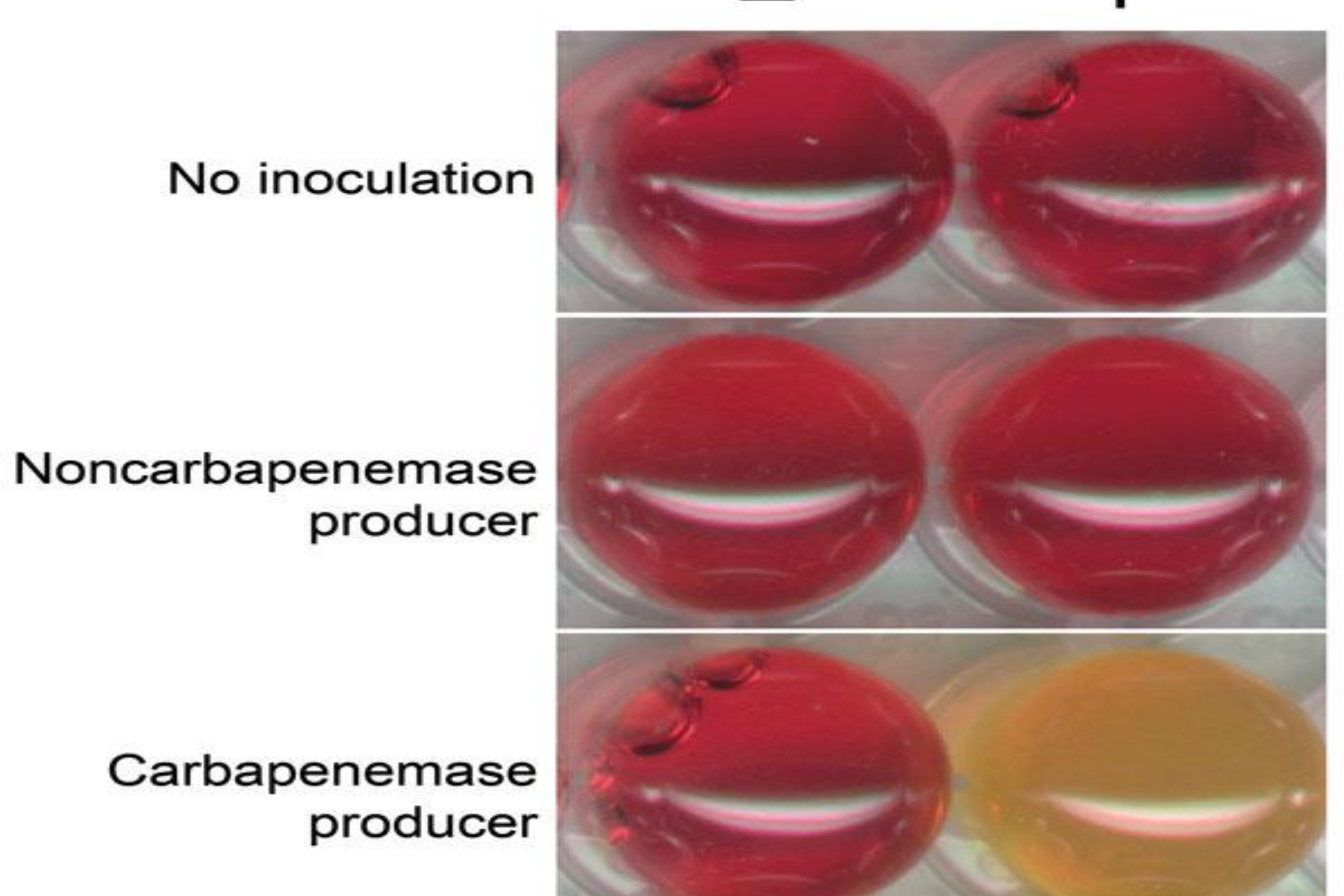
Tests for Carbapenemases

CARBA NP I and II

Carba NP Test for Detection of Carbapenemase Production in Enterobacteriaceae and *P. aeruginosa*[1]

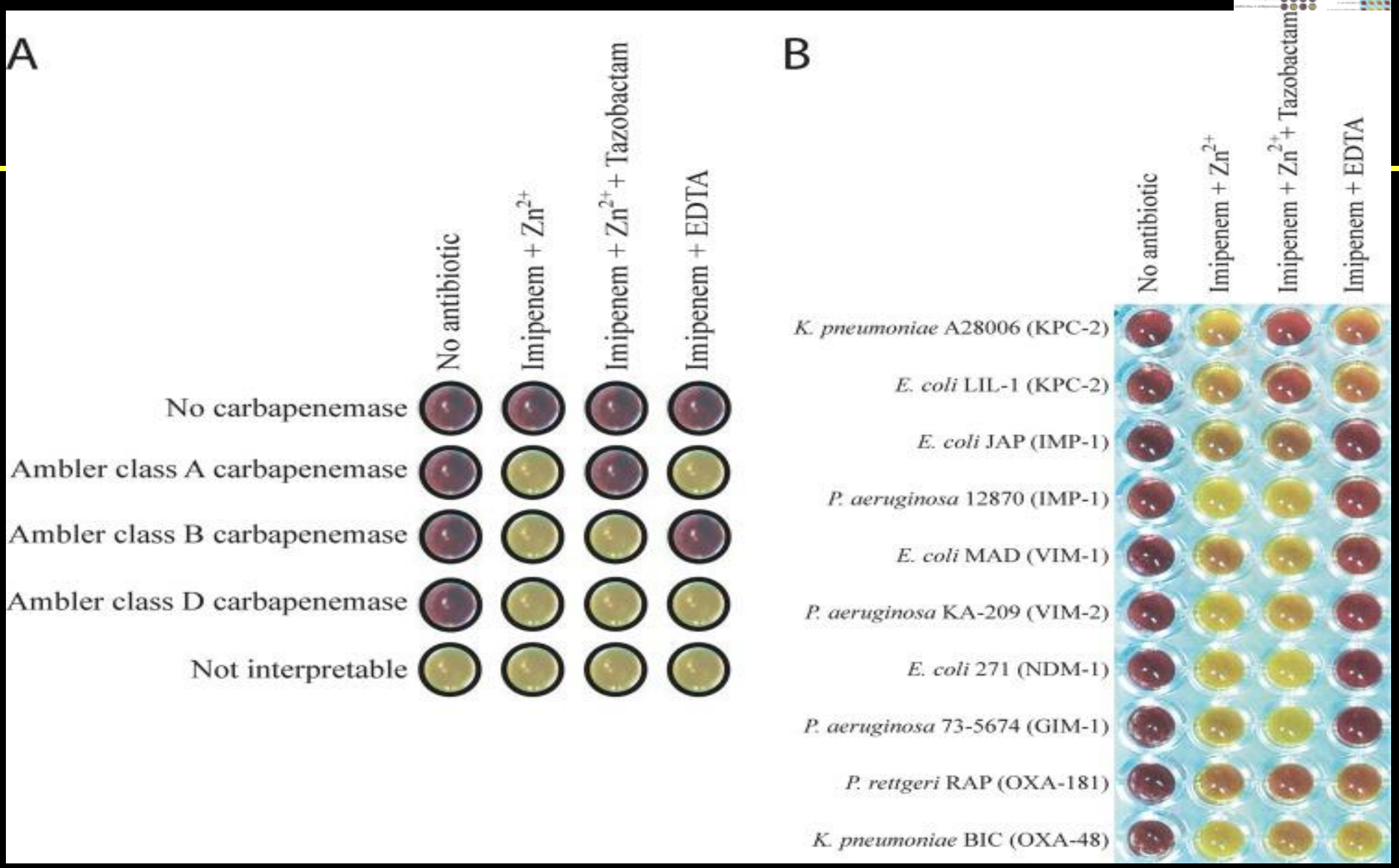
- Detects hydrolysis of imipenem
- Isolate suspended in TRIS-HCl lysis buffer, vortexed, incubated for 30 minutes, and centrifuged
- Supernate used in test wells, detect pH change
- Claimed it to be 100% sensitive and specific
  - 162 Class A, B and D Enterobacteriaceae (+)
  - 42 ESBLs. ampCs, porin mutations (-)
- A second study confirmed 100% specificity but lower sensitivity (72.5%)[2]
  - Sensitivity increased to 80% with a larger inoculum
  - OXA-48 accounted for 16/29 of the false negatives

1. Nordmann P, et al. 2012. EID 18: 1503 – 1506 2. Tijet, N. et al. 2013. AAC.57: 4578 - 4580


No inoculation
Noncarbapenemase producer
Carbapenemase producer

Carba NP II Test for Detection of Carbapenemase Production in Enterobacteriaceae

- **Use to determine the type of carbapenemase (Class A, B, or D)**
  - **Supernatant transferred to 4 wells of a microtiter plate respectively containing**
  - **Dilute phenol red solution with $ZnSO_4$, no antibiotic**
  - **Dilute phenol red solution with $ZnSO_4$ and imipenem**
  - **Dilute phenol red solution containing $ZnSO_4$, imipenem, and tazobactam**
  - **Dilute phenol red solution containing imipenem and EDTA**



Dortet L, Poirel L, Nordmann P. 2012. AAC. 56:6437-6440

Screening Cultures for CRE

- If you have CRE in your hospital, who, when, what and how should you screen?
- What do you do with screen results?
- Is it possible to eliminate CRE from the hospital?
- No FDA approved screening agar
  - Issues are both sensitivity and specificity

My Screening Method for CRE

- Stool sample in 5 ml TSB with 2 10µg disks of meropenem (4 µg/ml). Incubate overnight at 35
  - Plate 0.1 ml of above TSB broth on a MacConkey plate with 2 meropenem disks and incubate overnight at 35
  - Select colonies within zone of inhibition
  - ID by MALDI-TOF/Run susceptibility assay
- Can also plate stool directly on MAC w/disks

Resistance Issues in *Staphylococcus aureus*

Detecting Methicillin/Oxacillin Resistance in Staphylococci

Basics

Cefoxitin Disk Test for *mecA*-Mediated Resistance in *S. aureus and S. lugdunensis*

Agent	S	I	R
Cefoxitin (30 µg)	≥22	-	≤21

There are no oxacillin DD breakpoints for *S. aureus* and *S. lugdunensis*

Inoculum: direct colony method (0.5 McFarland)
MHA
Incubate at 35°C in ambient air
Read at 24 hours

CLSI M100-S25, 2015 Table 2C

Cefoxitin and Oxacillin MIC Breakpoints for *mecA*-Mediated Resistance in *S. aureus and S. lugdunensis*

Agent	S	R
Oxacillin	≤ 2	≥ 4
Cefoxitin	≤ 4	≥ 8

If both oxacillin and cefoxitin are tested and either one is resistant, report as resistant

Inoculum: direct colony method

Cation-supplemented MH broth with 2% NaCl and Oxacillin

Incubate at 35°C in ambient air

Read at 24 hours

CLSI M100-S25, 2015 Table 2C

Cefoxitin Disk Diffusion for *mecA*-mediated Resistance in Coagulase-Negative Staphylococci*

Agent	S	I	R
Cefoxitin (30 µg)	≥25	-	≤24

*Except *S. lugdunensis*

There are no CLSI oxacillin disk diffusion breakpoints

CLSI M100-S25, 2015 Table 2C

Oxacillin MIC Tests for *mecA*-Mediated Resistance in Coagulase- Negative Staphylococci except *S. lugdunensis*

Agent	S	R
Oxacillin	≤ 0.25	≥ 0.5

Note: There are no cefoxitin MIC breakpoints for CNS (except *S. lugdunensis*)

Inoculum: direct colony method

Cation-supplemented MH broth with 2% NaCl and Oxacillin

Incubate at 35°C in ambient air

Read at 24 hours

CLSI M100-S25, 2015 Table 2C

CLSI Comment 2015
CNS (except *S. epidermidis*)

"Oxacillin interpretive criteria may overcall resistance for some CNS because some non-*S. epidermidis* strains for which the oxacillin MICs are 0.5 to 2 µg/mL lack *mec*A. For serious infections with CNS other than *S. epidermidis*, testing for *mec*A or for PBP 2a or with cefoxitin disk diffusion may be appropriate for strains for which the oxacillin MICs are 0.5 to 2 µg/mL"

CLSI M100-S25, 2015 Table 2C

2014 CLSI Changes for Staphylococci (No changes to the 2015 Tables)

- **All cephalosporin breakpoints were removed except**
  - **Ceftaroline (1/2/4) for *S. aureus* (including MRSA)**
- **quinopristin/dalfopristin BPs for MRSA were removed**
- **Comment added for *mec*C**

  "Mechanisms of oxacillin resistance other than by *mec*A are rare and include a novel *mec*A homologue, *mec*C. MICs for strains with *mec*C are typically in the resistant range for cefoxitin and/or oxacillin; *mec*C resistance cannot be detected by tests directed at *mec*A or PBP 2a" (comment 3 M100-S25, table 2C)

Molecular Testing Pitfalls[1]

- **Homology between *mec*A in *S. aureus* and CNS in specimens with both MSSA and MR-CNS**
  - **Partially overcome by targeting a region that links the *S.aureus* SCC*mec* insertion site (SCC*mec-orfX* junction)**
  - **However, now detected *mec*A dropouts which resulted in false positives (up to 8%)**
  - **Modified test again to include *mec*A primers**
  - **Still possible to get false positives, but less likely**

Diekema and Pfaller. Clin Inf Dis. 56: 1615-1620. 2013

The Newest Challenge

***mec*C**

Case Reports: France[1]

- 67 yo male in France with knee joint infection (11/2007)
  - MRSA by disk diffusion
  - *mec*A negative by an in-house PCR and by the GenoType Staphylococcal test

1. wwwnc.cdc.gov/eid/article/18/9/11-1920_article.htm 2012

*Mec*C[1,2]

- First reported in 2006, mostly in Europe in animals and some cases in humans
- Found on SCC XI and originally noted as *mec*A$_{LGA251}$
  - 70% homology with *mec*A
- Issue: Resistant to methicillin by phenotypic assays (disk/MIC) but susceptible (not-detected) by molecular assays (*mec*A negative) and by PBP2a LA
- Vitek 2 study: 55/62 *mec*C strains OxS/CefoxR[3]

1. Garcia-Alvarez et al. 2011. Lancet Infect Dis. 11:595-603

2. Peterson et al. 2012. CMI. 19: E16-E22

3. Cartwright et al. 2013. J Clin Microbiol. 51:2732-2734

A Different Twist

Case Report

- **76 y/o male with prosthetic joint septic arthritis and bacteremia**
  - **Synovial fluid and 6 BC bottles positive for MSSA (Vitek 2): S to oxacillin, negative cefoxitin screen**
  - **OX MIC = 0.25 by BMD**
  - **Treatment plan: Vancomycin started (penicillin allergic) and prosthesis removed**
  - **12 more BC bottles positive for MSSA**
  - **Developed vertebral (T12-L1) osteomyelitis**
  - **Switched to nafcillin**

Case Continued

- 10 weeks of nafcillin w/ clinical response
- Re-presented with 2 weeks of back pain and a vertebral biopsy grew MRSA
  - MIC = 32 by BMD
- Treated with vancomycin, rifampin and nafcillin for 10 weeks with apparent cure
- However, MSSA and MRSA isolates were indistinguishable by PFGE and AP-PCR

"Emergence of methicillin-resistant *S. aureus* during treatment of methicillin-susceptible *S. aureus* bacteremia[1]

- Strains indistinguishable by PFGE and AP-PCR
- Mutated MSSA to MRSA by plating on media containing oxacillin
- All strains contained *mec*A
- DNA sequencing: MSSA isolates had an insertion sequence (IS *1181*) that was not present in the MRSA strain
- Oxacillin exposure promoted excision of the IS

1. Proulx, MK et al.2012 IDSA abstracts.Poster 834. San Diego

Vancomycin and Staphylococci

A Difficult Subject

CLSI Guidelines for Detecting Vancomycin Resistance in Staphylococci

- **MIC tests should be performed**
  - **Different breakpoints for *S. aureus* than for CNS**
  - **Send *S. aureus* isolates with an MIC ≥8 to a reference laboratory**
- **CLSI deleted vancomycin disk tests in 2009 because**
  - **Disk Diffusion tests do not differentiate vancomycin susceptible strains from vancomycin intermediate strains**
  - **Disk test does not differentiate among S, I and R in CNS**
  - **Disk test will detect *S. aureus* isolates containing *van*A**

CLSI M100-S25 2015, Table 2C

CLSI *S. aureus* Vancomycin Breakpoints

	Old	New
Susceptible	≤4	≤2
Intermediate	8-16	4-8
Resistant	≥32	≥16

CLSI M100-S25 2015

The Emergence of VISA (Vancomycin-Intermediate *S. aureus*)

- **France 1995: 2 year old girl with leukemia and a central line associated bacteremia**
  - **Treated with surgical drainage and quinupristin-dalfopristin (survived)**
- **Japan 1996: 4 month old that was treated for 29 days with vancomycin. Initial isolate was susceptible, subsequent isolate had vancomycin MIC = 8 ug/ml**
  - **Treated successfully with aberkacin and amp/sulbactam**
- **2015: Numerous reports of VISA and hVISA**

Heteroresistant Vancomycin-Intermediate *S. aureus* (hVISA)

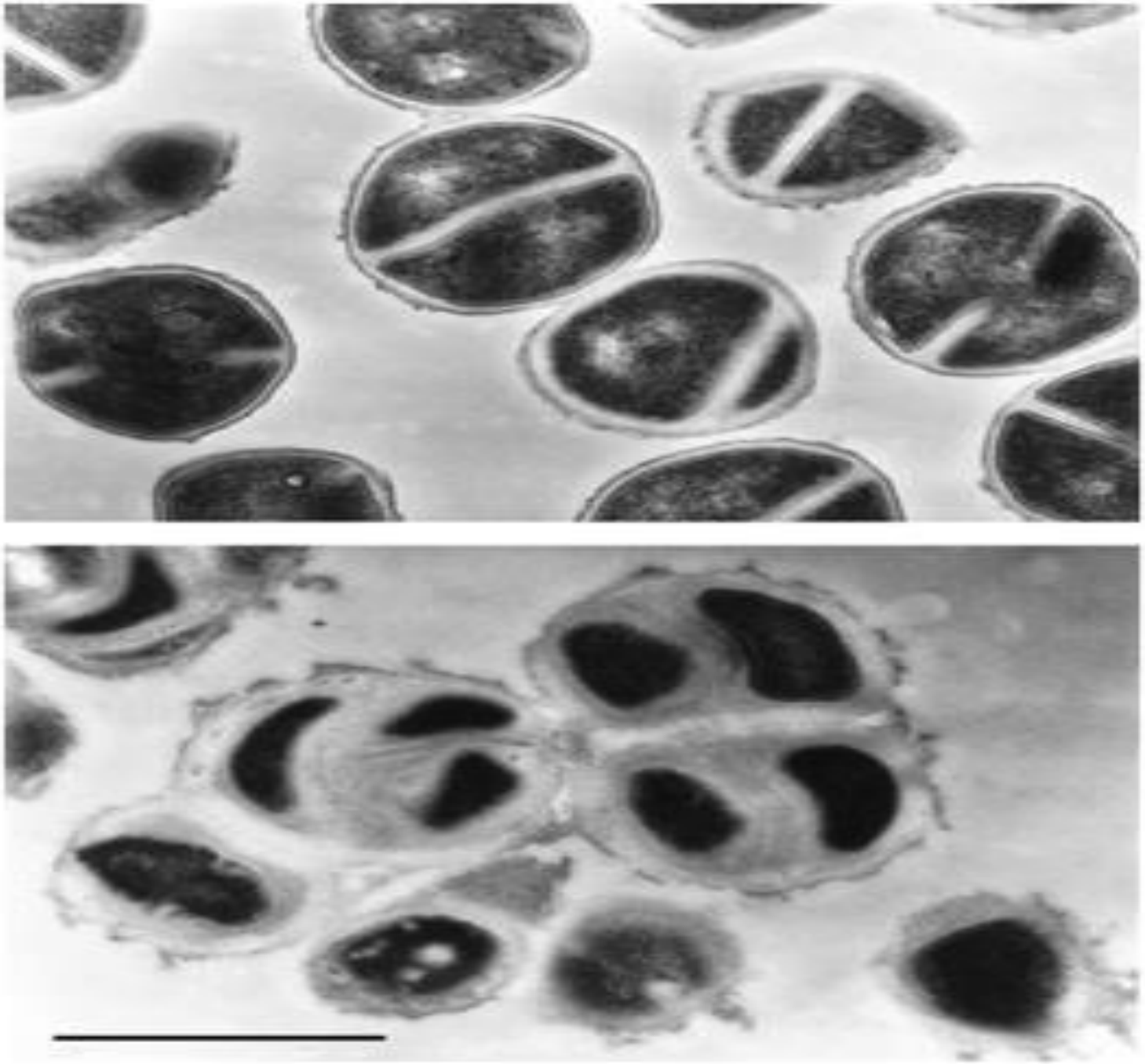
- Represents subpopulations of less susceptible organisms within a population of susceptible organisms (10^{-5} to 10^{-6})
- Not detected or inconsistent detection by standard MIC tests[1-4]
- Difficult to differentiate strains with MICs between 2-4[4]

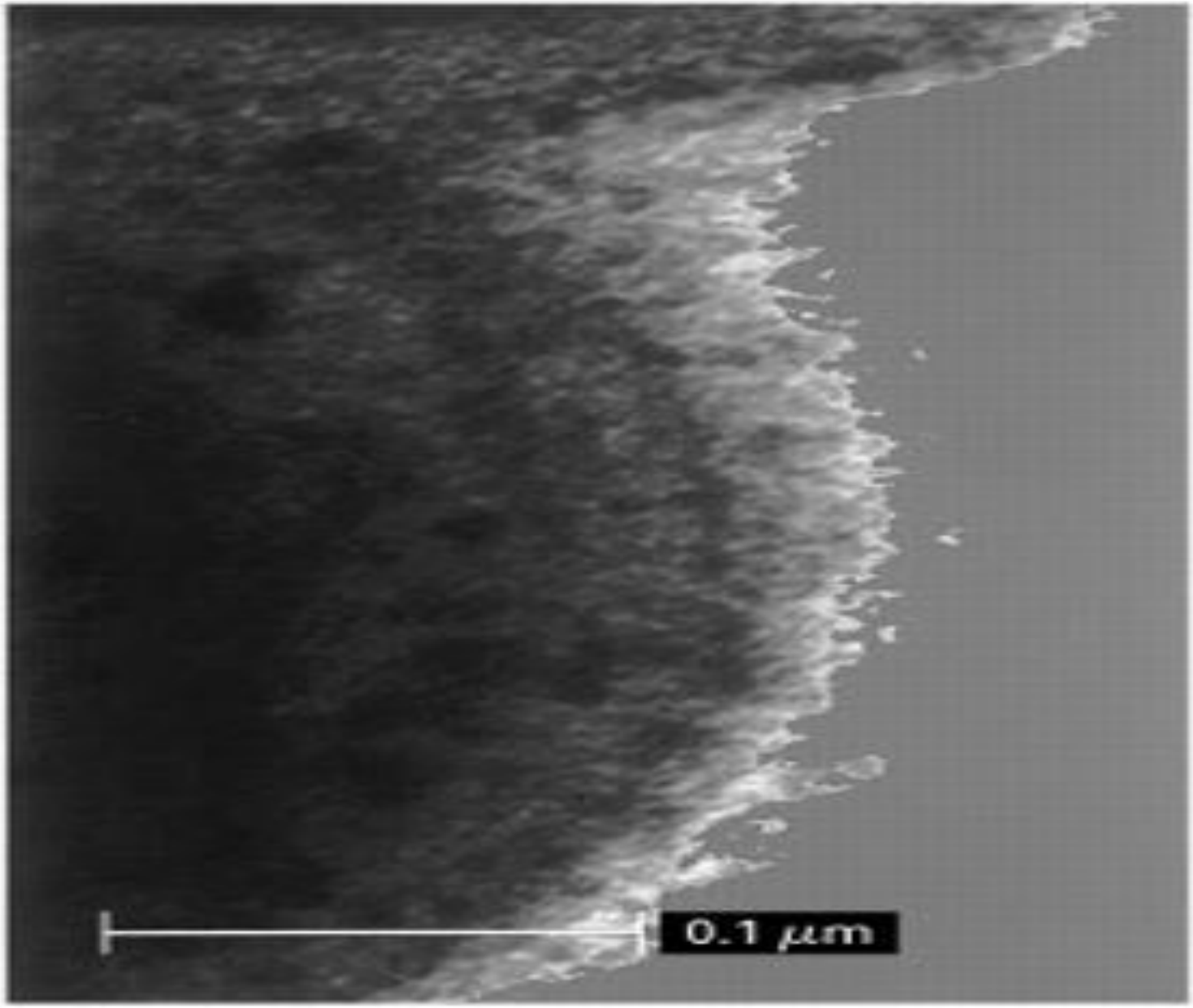
1. Prakash et al. AAC. 52:4528. 2008
2. Swenson, J et al. ICCAC abstracts. 2008
3. Maor,Y. et al. JID. 199:619-624. 2009
4. Deresinski, S. JID. 199: 605-609. 2009

VISA: Mechanism of Resistance

- Appears to involve alterations in the bacterial cell wall
- Glycopeptide molecules may be captured at a site distant from cell wall synthesis
- Increase in cell wall turnover that results in an excess of non cross-linked D-Ala-D-Ala
- Vancomycin disappears from culture media
- Thickened cell wall in the presence of vancomycin
- Large amount of extracellular matrix material on the outer cell wall

Sieradzki et al. NEJM 340 :517-523.1999




0.1 μm

Vancomycin MICs by 3 Automated Methods and by E-Test[1]

- 200 strains of MRSA
- Reference method: BMD
- Test methods
  - MicroScan
  - Vitek 2
  - Phoenix
  - E-test

1. Rybak, MJ et al. J Clin Microbiol. 51: 2077-2081. 2013

Results[1]

- **Agreement with BMD**
  - **Phoenix**
    - **66.2% agreement; likely to under call MIC of 2.0**
  - **MicroScan**
    - **61.8% agreement; prompt likely to overcall an MIC of 1.0**
    - **turbidity method more accurate than prompt method**
  - **Vitek 2**
    - **54.3% agreement; likely to under call an MIC of 2.0**
  - **E-Test**
    - **36.7% agreement; MIC 1-2 dilutions higher than BMD**

1. Rybak, MJ et al. J Clin Microbiol. 51: 2077-2081. 2013

Vancomycin MICs by E-Test, MicroScan and Phoenix

MIC in micrograms/ml

Method	0.5	1.0	2.0	4.0
E-Test	0	101	49	0
MS-TU	1	134	14	1
MS-PR	1	76	71	2
Phoenix	83	65	3	0
BMD(CLSI)	1	138	11	0

100 Clinical Isolates of MRSA and 50 MSSA

Riedel, S. et al. 2014. JCM. 52: 2216-2122

Daptomycin MICs by E-Test, MicroScan and Phoenix

MIC in micrograms/ml

Method	≤ 0.5	1.0	≥ 2.0
E-Test	141	7	2
MS-TU	145	3	2
MS-PR	103	44	3
Phoenix	144	4	2
BMD(CLSI)	144	4	2

100 Clinical Isolates of MRSA and 50 MSSA

Riedel, S. et al. 2014. JCM. 52: 2216-2122

MRSA Guidelines How Should Vancomycin MICs Be Used to Guide Therapy?[1]

- **If the vancomycin MIC is ≤2 µg/mL, the patient's clinical response should determine the continued use of vancomycin, independent of MIC (A-III)**
  - **Because current susceptibility testing methods are unable to reliably distinguish MICs of 1 µg/mL from MICs of 2 µg/mL, the Panel recommends evaluation of the patient's clinical and microbiologic response along with MIC results when making therapy decisions**
  - **If clinical and microbiologic response to vancomycin, then continue with close follow-up**
  - **If no clinical or microbiologic response despite adequate debridement and removal of other foci of infection, an alternative to vancomycin should be considered regardless of MIC**
- **For isolates with a vancomycin MIC >2 µg/mL (eg, VISA or VRSA), the panel recommends using an alternative to vancomycin (A-III)**

1. Liu et al. 2011Clin Inf Dis.;52:385-92

What About VRSA?

- **VRSA had been made in the laboratory by transconjugation of the *vanA* gene from *E. faecalis* into *S. aureus***
- ***When would this happen in humans?***

NOW

Noble et al. FEMS Microbiol. Lett. 93: 195-198. 1992

VRSA

- **July 5, 2002 MMWR: *S. aureus* fully resistant to vancomycin**
  - **40 y/o female w/ diabetes, PVD and renal failure**
  - **First isolate to naturally acquire the van*A* gene from *E. faecalis*. The patient had both VRE and VRSA**
  - **MIC was >1024**
- **13 reported cases in US as of 10/2015**
  - **8/13 in Michigan**
  - **Do not appear to be clonally related**

VRSA: Why So Few?

- Many patients are colonized and infected with VRE and MRSA
- The vanA gene from *E. faecalis* is on a transposon which is on a plasmid and may require a special signal for conjugation[1]
  - With the first VRSA, the vanA gene integrated into the chromosome, but the plasmid was enzymatically degraded[2]
- *S. aureus* has an enzyme system that protects itself from foreign DNA[3]
- Strains have been isolated that have mutations that allow foreign DNA to integrate (termed "hyperrecepient strains")[4]
  - Hyperrecipient strains may be necessary for gene transfer from enterococci

1. Clewell DB, et al. *J Bacteriol*. 1985;162:1212-1220.
2. Flannagan SE, et al. *AAC*. 2003;47:3954-3959.
3. Waldron DE, and JA Lindsay. *J Bacteriol*. 2006;188:5578-5585.
4. Sung JML and Lindsay JA. *AAC*. 2007;51:2189-2191.

Transferable Vancomycin Resistance in a Community-Associated MRSA Lineage[1]

- CA MRSA strain from blood acquired *van*A during treatment (Brazil)
- Conjugative plasmid (pBRZ01) carrying *van*A gene cluster identified and transferred to other staphylococci
- This could be the next big public health issue in microbiology and ID

1. Rossi, F. et al. 2014. NEJM.370:1524-1531

Although the ASM is Providing Lunch Let Me Suggest A Market Just Down the Road in Dorchester