Interacting with your laboratory colleagues

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GA CRE Collaborative

Learning Session 1

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National Center for Emerging and Zoonotic Infectious Diseases
Division of Healthcare Quality Promotion

Presentation Objectives

- Basic terms used in the microbiology lab
- Understand carbapenem-resistance in gram-negative bacteria
- Describe laboratory testing for carbapenem-resistance
- Examine your process for communicating with the laboratory

Disclosure – Dr. Stone is NOT a microbiologist

Acknowledgement – Dr. Burd, Director of Clinical Microbiology at Emory University Hospital provided content for many of these slides

Microbiology 101: Identification

Growing the bacteria

- Traditional culture
  - Uses gram stain, biochemical reactions for identification
- Selective culture media
    - Example: CHROMagar

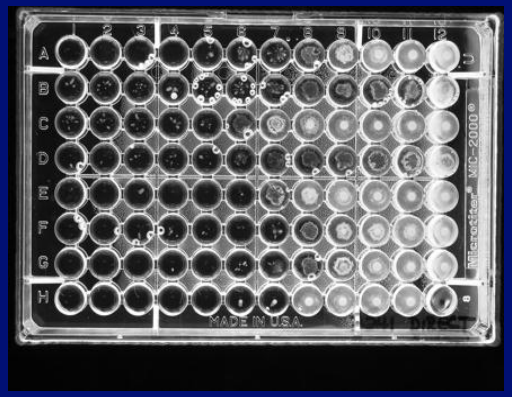
Examining parts of the bacteria

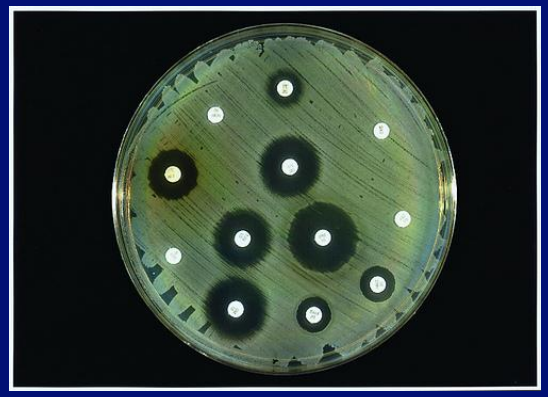
- Molecular diagnostic tests
  - Identify specific fragments of DNA/RNA of organisms
    - Nucleic acid amplification tests (NAAT);Polymerase chain reaction (PCR)
- Matrix-assisted laser desorption/ionization (MALDI-TOF)
  - Very new technology: Uses mass spectrometry to identify bacteria based on weight and charge of ions

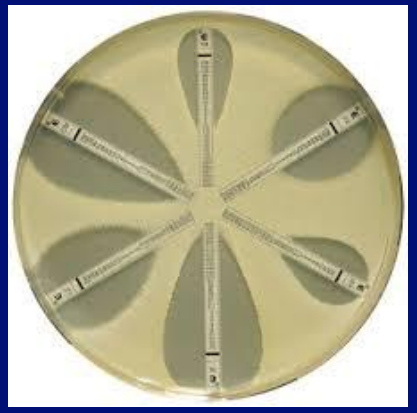
Microbiology 101: Susceptibility

Testing the growth in the presence of antibiotic

- Determining the minimum inhibitory concentration (MIC) – lowest amount of drug needed to stop growth
- Broth micro-dilution, Disk diffusion, E-test strips







Identifying resistance genes

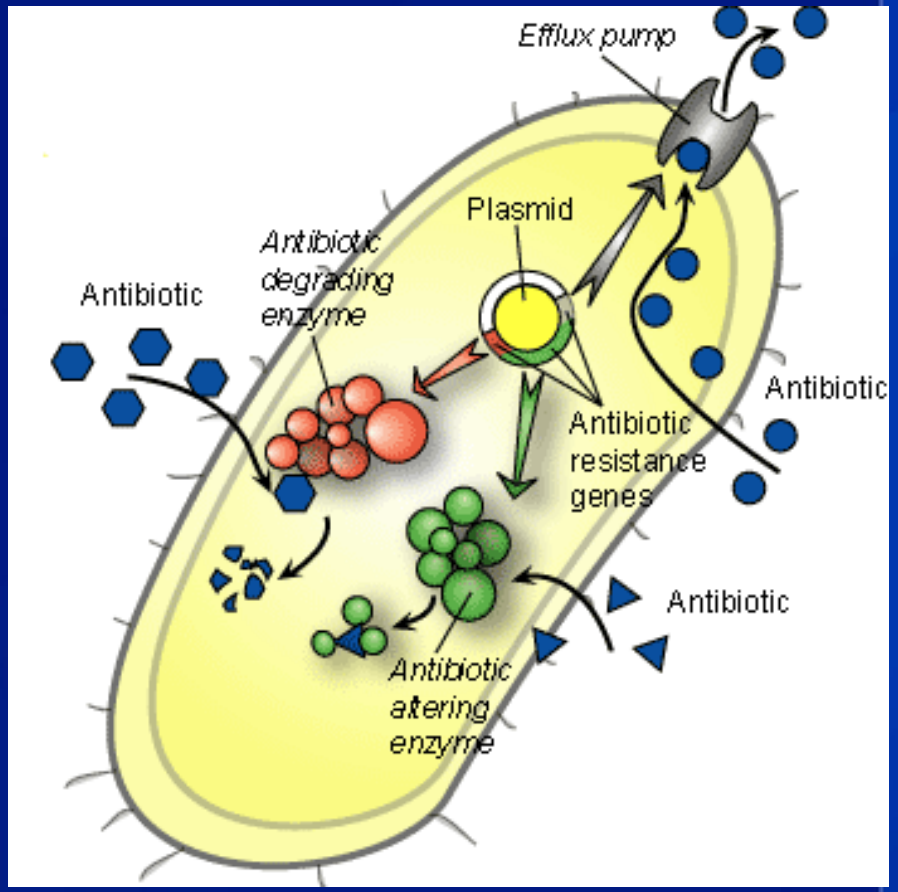
- Molecular diagnostic tests – detect presence of specific resistance genes (NAAT, PCR)

Microbiology 101: Automated testing

- Systems with identification and susceptibility in one platform
  - Special growth panels contain biochemicals for identification and antibiotics for susceptibility testing
    - Bacteria of interest are innoculated onto panels and placed into system
  - Computer will identify organism and susceptibility interpretation
    - Uses pre-programmed algorithms based on growth patterns of bacteria on the panel
  - Example systems (trade names): Microscan, Walkaway, VITEK 2, Phoenix, Sensititre

Mechanisms of antibiotic resistance

- Production of proteins that destroy antibiotics
  - Beta-lactamases
  - Cephalosporinases
  - Carbapenemases
- Change their cell structure
  - Block s binding and function of antibiotics
- Reduce exposure
  - Pump antibiotics out
  - Increase cell barriers to block entry



http://bioinfo.bact.wisc.edu/themicrobialworld/bactresanti.html

Case scenario

- 70 year old admitted from hospital to nursing home
  - Treated with Ceftriaxone for catheter-associated UTI x7 days before transfer
  - Catheter still in place recently transferred
- Repeat urine culture ordered by MD prior to removing catheter
  - Organism: E. coli, $>10^5$ cfu

Drug	Result
Amikacin	Susceptible
Ampicillin	Resistant
Amp/Sulbactam	Resistant
Aztreonam	Resistant
Cefazolin	Resistant
Cefepime	Resistant
Ceftazidime	Resistant
Ceftriaxone	Resistant
Cefuroxime	Resistant
Gentamicin	Susceptible
Levofloxcin	Resistant
Meropenem	Susceptible
Piperacillin/Tazobactam	Resistant
Tobramycin	Susceptible
Trimethoprim/Sulfa	Resistant

Remember the good old days…

Cephalosporin resistance in gram-negative bacteria

- Some organisms had resistance genes within their chromosomes (Example: AmpC)
  - Bacteria already had the capability to be resistant
  - Resistance was uncovered with overexpression of the gene
  - *Consider in bugs like Serratia, Pseudomonas, Acinetobacter*
- Other organisms acquired resistance genes through mobile elements
  - Example: Extended spectrum Beta-lactamases (ESBLs)
  - *Consider in E. Coli, Klebsiella*
- Now we see both types of cephalosporin-resistance expressed in different bacteria
  - Does mechanism of resistance matter?

Changes in defining cephalosporin susceptibility (2010)

- Changing the MICs redefines the susceptibility of bacteria
- From a laboratory testing perspective, lowering the MIC that defines "susceptible" should increase identification of resistance

	Old Breakpoints			New Breakpoints		
	MIC (µg/ml)			MIC (µg/ml)		
	S	I	R	S	I	R
Cefazolin	≤ 8	16	≥ 32	≤1	2	≥ 4
Ceftriaxone	≤ 8	16-32	≥64	≤1	2	≥ 4
Ceftazidime	≤ 8	16	≥ 32	≤4	8	≥ 16
Cefepime	≤ 8	16	≥ 32	≤ 8	16	≥ 32

Case scenario #2

- 70 year old admitted from hospital to nursing home
- Had complicated history including surgery, ICU care, ventilator-weaning
  - On transfer, has PICC line, tracheostomy, PEG tube, urinary catheter and large sacral pressure ulcer
- MD sends culture from tracheostomy secretions
  - Organism: *Klebsiella pneumoniae*, $>10^5$ cfu

Drug	Result
Amikacin	Intermediate
Ampicillin	Resistant
Amp/Sulbactam	Resistant
Aztreonam	Resistant
Cefazolin	Resistant
Cefepime	Resistant
Ceftazidime	Resistant
Ceftriaxone	Resistant
Cefuroxime	Resistant
Gentamicin	Resistant
Levofloxcin	Resistant
Meropenem	Resistant
Piperacillin/Tazobactam	Resistant
Tobramycin	Resistant
Trimethoprim/Sulfa	Resistant

Carbapenem-resistance in gram-negative bacteria

- Carbapenems are reserved for severe, complicated infections with multiple and often resistant bacteria
  - Recall: "Extremely broad-spectrum"
- Resistance significantly limits treatment options for life-threatening infections
  - No new antibiotics in development for gram-negative bacteria
- Emerging resistance mechanisms can be spread
  - Carbapenemases are found on mobile genetic elements
- Detection of carbapenemases and implementation of infection control practices are necessary to prevent spread

Carbapenem-resistance: Mechanisms

Enterobacteriaceae	Cephalosporinase + porin loss
	Carbapenemase
P. Aeruginosa	Cephalosporinase + porin loss
	Up-regulated efflux pump
	Carbapenemase
Acinetobacter spp.	Cephalosporinase + porin loss
	Carbapenemase

Slide courtesy of Dr. Eileen Burd, Emory University Hospital

Types of carbapenemases

Classification	Enzyme	Most Common Bacteria
Class A	KPC, SME, IMI, NMC, GES	Enterobacteriaceae (rare reports in *P. aeruginosa*)
Class B (metallo-β-lactamase)	NDM, IMP, VIM, GIM, SPM	*P. aeruginosa* Enterobacteriaceae *Acinetobacter* spp.
Class D	OXA-48	*Acinetobacter* spp. (reports in Enterobacteriaceae)

Slide courtesy of Dr. Eileen Burd, Emory University Hospital

Why focus on carbapenemases?

- The genetic material creating carbapenemases sits on highly mobile elements
  - These resistance elements can be shared between different bacteria very easily
  - Similar to concern with ESBL spreading cephalosporin-resistance
- Two carbapenemases getting lots of attention
  - *Klebsiella pneumoniae* carbapenemase (**KPC**)
  - New Delhi metallo-beta-lactamase **(NDM-1)**
- Identifying/containing bacteria which produce carbapenemase will *prevent the spread of resistance to other people and other organisms*

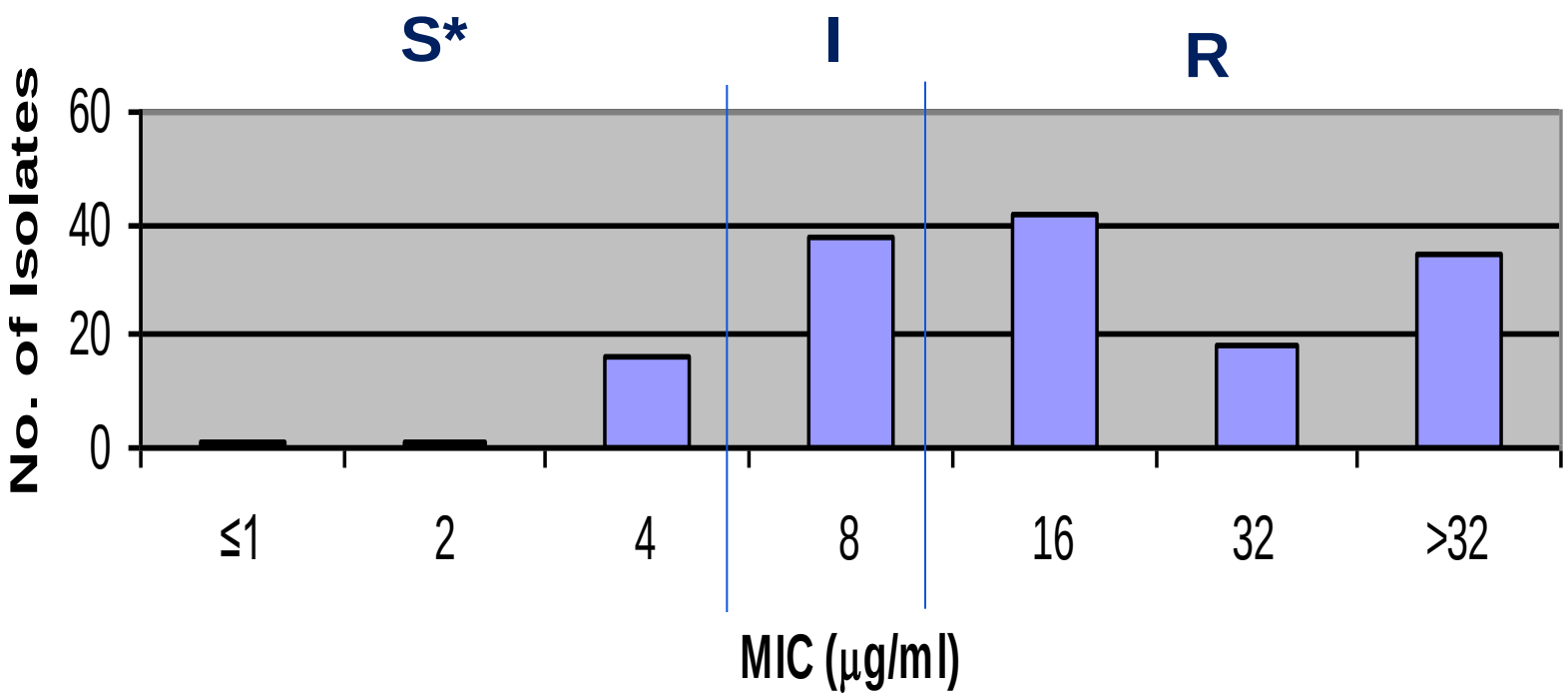
Can laboratories identify carbapenemases?

- Recall: Labs look for susceptibility to carbapenems by manual or automatic testing methods

Challenges:

- Identification of carbapenem-resistance varies by which carbapenem is used for susceptibility testing
- Low-levels of carbapenem resistance that may not be detected by automated testing
- Even if carbapenem resistance is detected – Not all carbapenem-resistance means the bacteria produces a carbapenemase

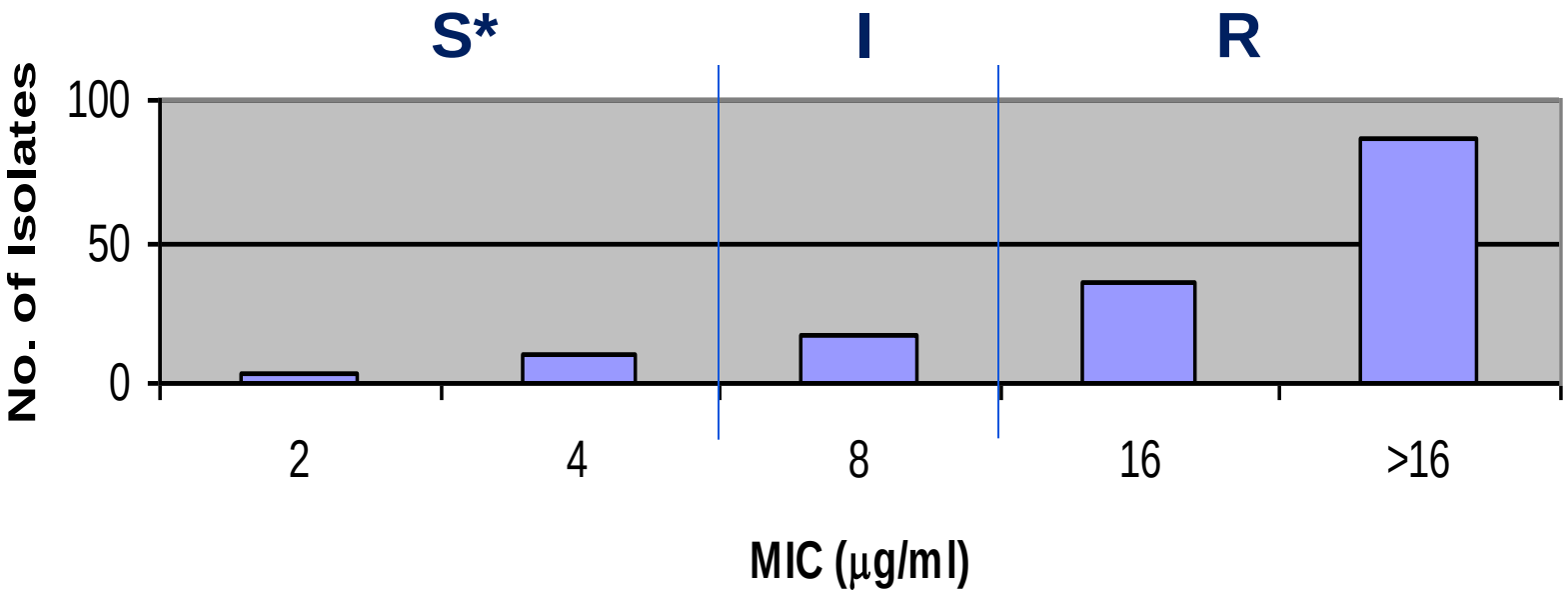
Susceptibility of KPC-Producers to Imipenem



*12% of isolates test susceptible to imipenem

Slide courtesy of Dr. Eileen Burd, Emory University Hospital

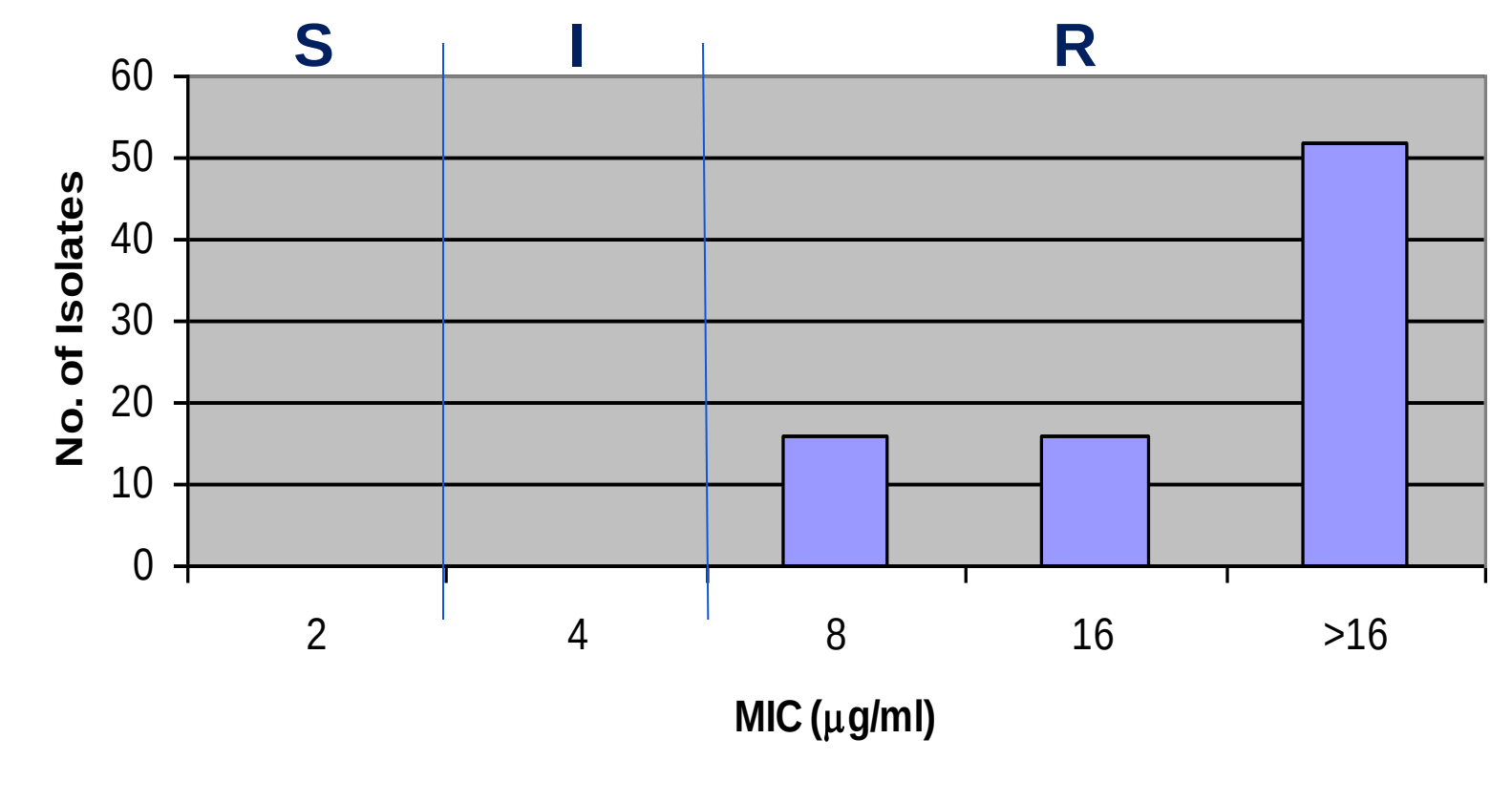
Susceptibility of KPC-Producers to Meropenem



*9% of isolates test susceptible to meropenem

Slide courtesy of Dr. Eileen Burd, Emory University Hospital

Susceptibility of KPC-Producers to Ertapenem



None of the isolates test susceptible to ertapenem

Slide courtesy of Dr. Eileen Burd, Emory University Hospital

Can Carbapenem Susceptibility of "I" or "R" detect KPC-producers?

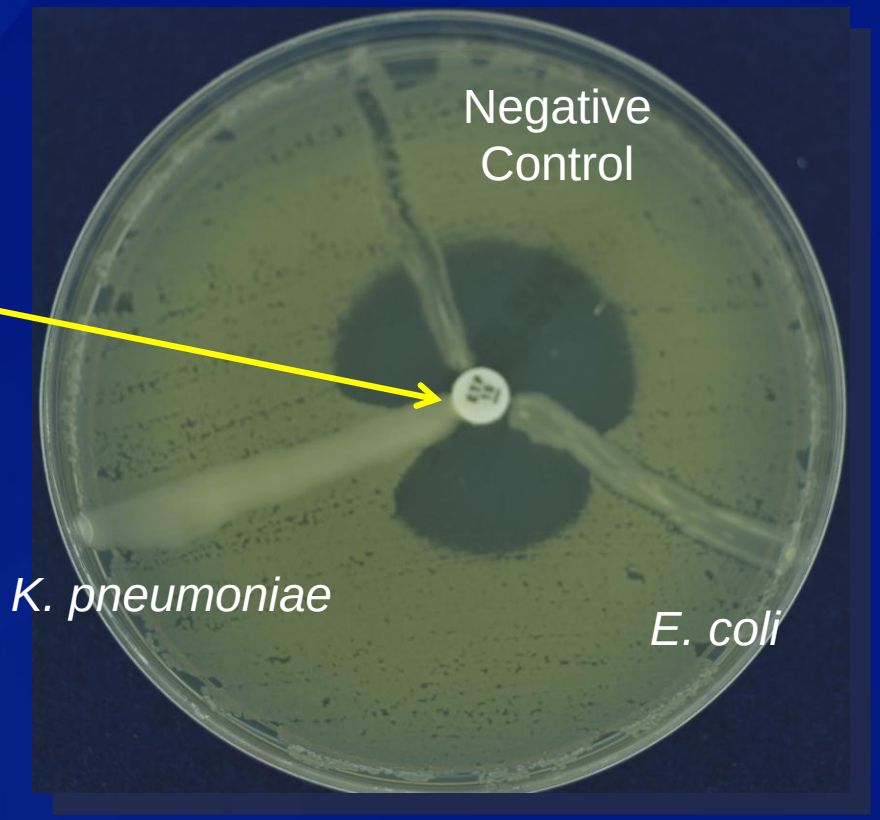
Method	Sens/Spec (%) for Detection of KPC-mediated R		
	Imipenem	Meropenem	Ertapenem
Ref BMD	94/93	94/98	97/89
Disk Diffusion	42/96	71/96	97/82
Etest	55/96	58/96	90/84
Vitek Legacy	55/96	52/98	N/A
Vitek 2	71/98	48/96	94/93
MicroScan	74/96	84/98	100/89
Phoenix	81/96	61/98	N/A

Low sensitivity = might miss true KPC;
Low specificity = might over-call carbapenem resistance.

Anderson KF et al., 2007. J. Clin. Microbiol. 45:2723-5.

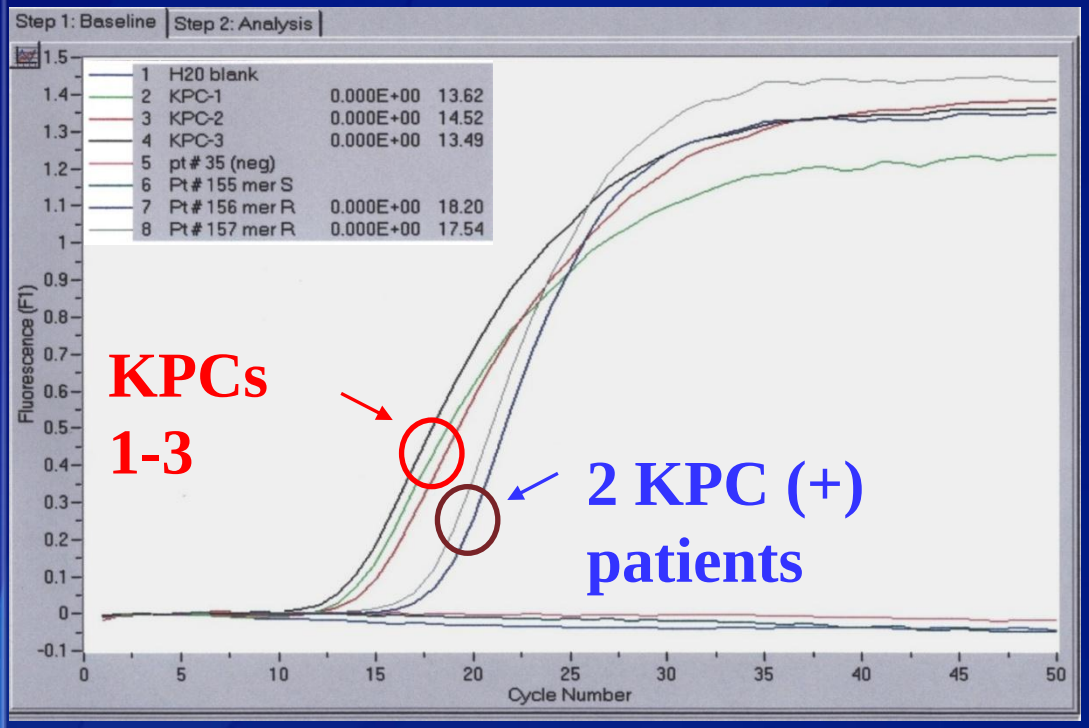
Confirming carbapenemase by growth: Modified Hodge test

- Mueller Hinton Agar plate
  - Lawn of E. coli ATCC 25922
- Carbapenem disc in center
- Instead of a clear zone of inhibition, the zone gets distorted when carbapemase is present



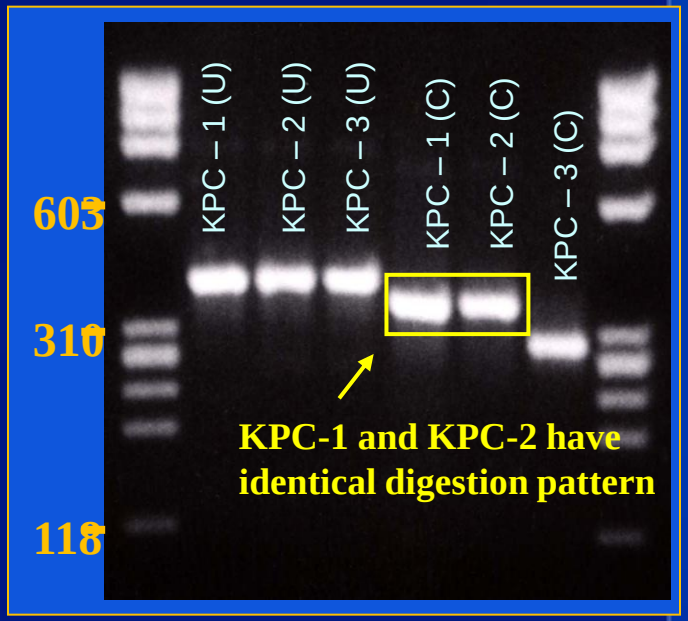
Described by Lee K et al. *Clinical Microbiology and Infection* 7: 88-102, 2001.

Confirming carbapemase by molecular detection methods



bla$_{KPC}$ PCR
- Forward primer 5'-TCTGGACCGCTGGGAGCTGG-3'
- Reverse primer 5'-TGCCCGTTGACGCCCAATCC-3'
- Probe 5'FAM-CGCGCGCCGTGACGGAAAGC-TAMRA3'

KPC Isoenzyme Differentiation



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Changes in defining carbapenem susceptibility (2010-2012)

- Changing the MICs will redefine susceptibility of bacteria
- From a laboratory testing perspective, lowering the MIC that defines "susceptible" should increase identification of resistance

	Old Breakpoints			New Breakpoints		
	MIC (µg/ml)			MIC (µg/ml)		
	S	I	R	S	I	R
Eratpenem	≤ 2	4	≥ 8	≤0.5	1	≥ 2
Imipenem	≤ 4	8	≥16	≤1	2	≥ 4
Meropenem	≤ 4	8	≥ 16	≤1	2	≥ 4
Doripenem	--	--	--	≤1	2	≥ 4

What does it all mean??

- Many mechanisms can cause carbapenem-resistance in gram-negative bacteria
- Microbiology labs may use different strategies for detecting carbapenem-resistance
  - Reliable detection may vary by testing method being used
- Labs may NOT do the additional confirmatory testing to determine if resistance is from a carbapenemase
  - Requires additional knowledge, supplies/resources, time and technology
- Understanding the methods/capacity of your laboratory is a critical step in determining the burden of carbapenem-resistance in your facility
  - May be over or under-estimated

Starting the conversation with your lab

- Talk with the director of microbiology for your laboratory
  - Share your interest in understanding the carbapenem resistance in gram-negative bacteria identified in your facility
- Ask what methods are used for identification and antibiotic susceptibility
  - Is it an automated method? Can they flag organisms with carbapenem-resistance?
- Ask whether they can to perform "confirmatory" testing for carbapenemase-production (e.g., modified Hodge)
  - Could this be done if requested?
- Discuss a strategy for notifying infection prevention when a carbapenem-resistant bacteria is identified

Thank you!!

Email: nstone@cdc.gov with questions/comments

For more information please contact Centers for Disease Control and Prevention

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