Identifying CRE Infections and Interpreting CRE Lab Results

Infection Prevention Symposium:

Carbapenem-Resistant Enterobacteriaceae (CRE)

Philadelphia, PA

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Objectives

- Review mechanisms of multidrug and carbapenem resistance among Enterobacteriaceae
- Review molecular types of carbapenemases, global distribution, prominent types in US and PA
- Discuss appropriate diagnostic lab tests (advantages & limitations) for the detection of carbapenemases in clinical samples and in surveillance samples
  - Commercial MIC testing systems: automated & manual
  - Utilization of Modified Hodge Test
  - Phenotypic inhibiton tests for carbapenemases
  - Screening media: ertapenem broth screen, chromogenic agar
- Explain how to interpret and report CRE results

Question #1

What are the major mechanisms of antimicrobial resistance and specifically carbapenem resistance among Enterobacteriaceae?

Mechanisms of Antimicrobial Resistance Among Gram-Negative Pathogens

I. Reduced Drug Accumulation

- **Down-reg outer membr porin prot (OmpF35 – carb-R w AmpC in *K pne*)[a]**
- **Upreg of efflux pumps (AcrAB-TolC – cefurox-R in *E coli*)[b]**

II. Inactivating Enzymes

- **Extended spectrum beta-lactamases (SHV-5 – 3g ceph-R in *K pne*)[c]**
- **AmpC cephalosporinases (CMY-1 (pl) – 3g ceph-R in *K pne & E coli*)[c]**
- **Carbapenemases (KPC-2 (plasmid) – pen, ceph, carb-R in *K pne*)[c]**
  (SME-3 (chromosomal) – *S marcescens*)[d]

III. Alteration of Drug Targets

- **Altered penicillin-binding protein (carb-R in *P mirabilis*)[e]**
- **Modif PO_4 grp of lipid A (colistin/polymyxin -R in *E coli & P aer*)[a,e,f]**

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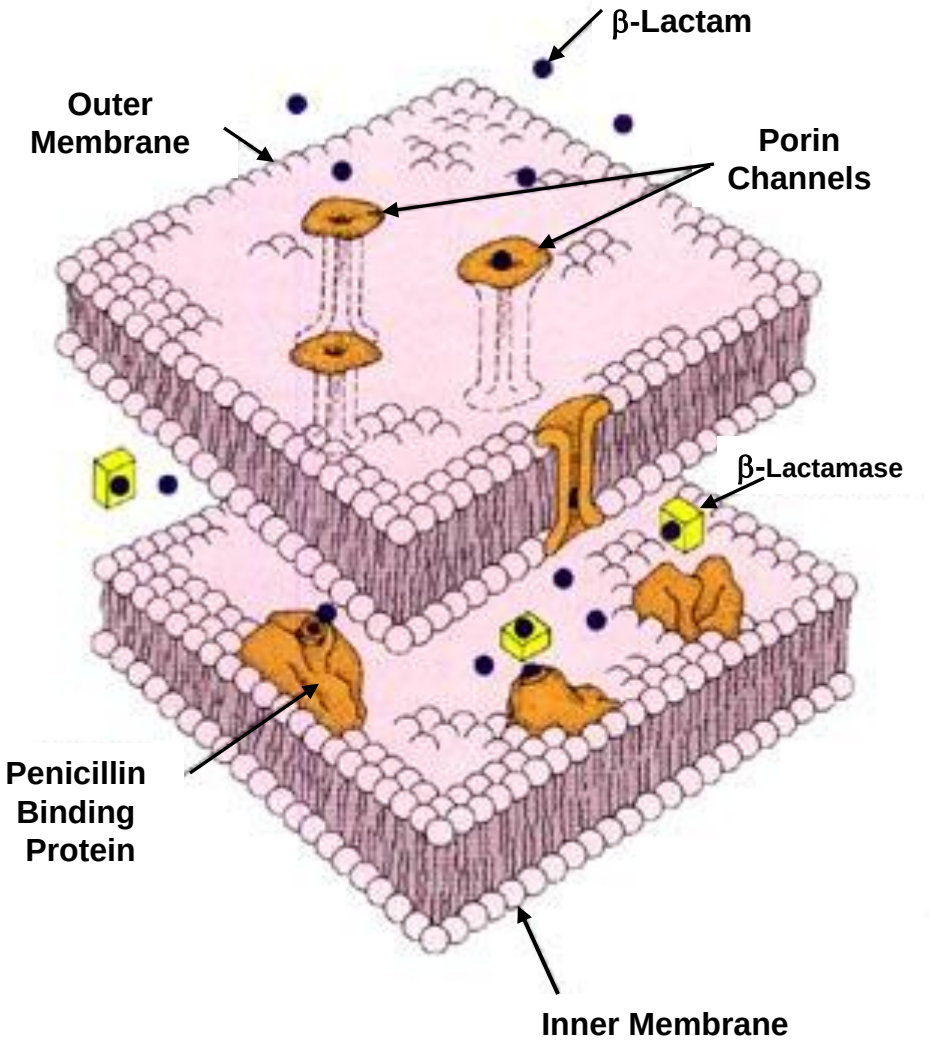
a: Rice et al. In *Man Clin Micro.* 2007, Chap 71. b: Piddock. *Clin Micro Rev*. 2006;19:382-402.
c: Jacoby et al. *N Engl J Med*. 2005;352:380-91. d: Queenan et al. *Clin Micro Rev* 2007;20:440-58
e: Villar et al. *JAC*. 1997;40:365-70. f: Li et al. *IJAA*. 2005;5:11-25.

Gram-Negative Bacterial Cell Membranes



Reproduced from Medical Microbiology A at the University of Tasmania. Available at: http://www.hls.utas.edu.au/teaching/micro/mma.fb/mma.fb.10.html. Accessed December 2, 2008. .

Carbapenem Beta-Lactamases & Carb-R

Class	Common β-lactamase type	Organisms
A	β-lactamase w serine at active site (KPC 2, 3, 4) also TEM, SHV, CTX-M ESBLs + porin loss	*Klebsiella, E coli, Enterobacter*
B	metallo-β-lactamase (Zinc at active site)(VIM, IMP, NDM-1)	*Klebsiella, Acinetobacter*
C	AmpC-like + porin loss (plasmid: CMY, FOX, DHA)	*Klebsiella, E coli, Enterobacter*
D	Oxacillinase (OXA-48)	*Klebsiella*

Queenan & Bush. *Clin Micro Rev.* 2007;20:440-58
Rahal. *Critical Care*. 2008;12 (S4):S5-11.

Question #2

What is the global distribution of carbapenemase- producing Enterobacteriaceae (CRE) and which enzymes are found in isolates from the US and from Pennsylvania?

Geographic Locations of Carbapenemases

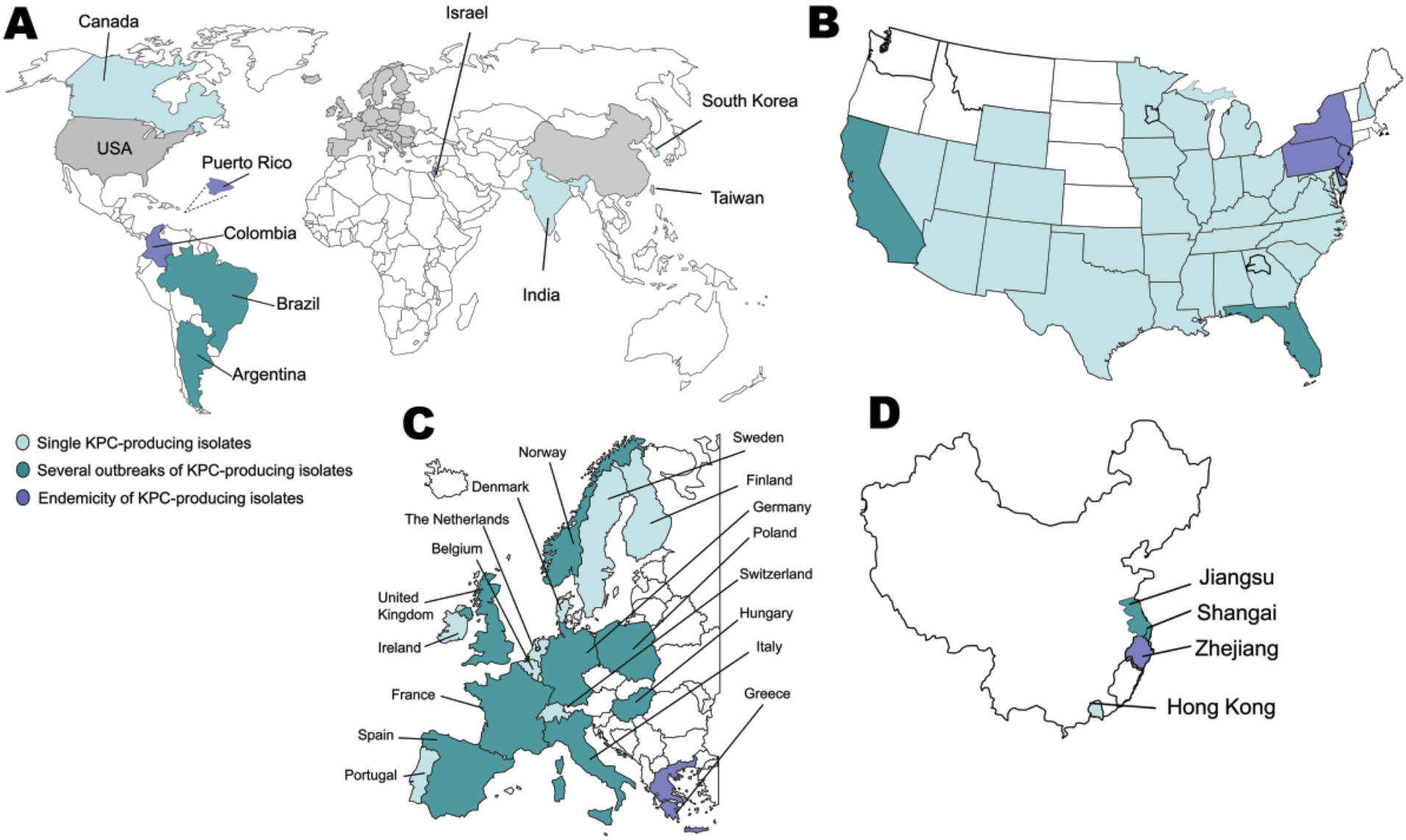
Class: enzyme	Common Locations	Types identified
A: KPC	Israel, Greece, Europe, US (44 st), PR, S America, India, Eastern China	KPC 2-15
B: NDM	India, Pakistan, Europe, China, US (11 st)	NDM 1-10
B: VIM	Turkey, Greece, Europe, US (2 st)	VIM 1-39
B: IMP	Japan, Taiwan, Singapore, Australia, US (CA)	IMP 1-45
D: OXA	Turkey, W Europe, N Africa, India, US (5 st)	OXA 1-364

Queenan & Bush. *Clin Micro Rev.* 2007;20:440-58

Rahal. *Critical Care.* 2008;12 (S4):S5-11.

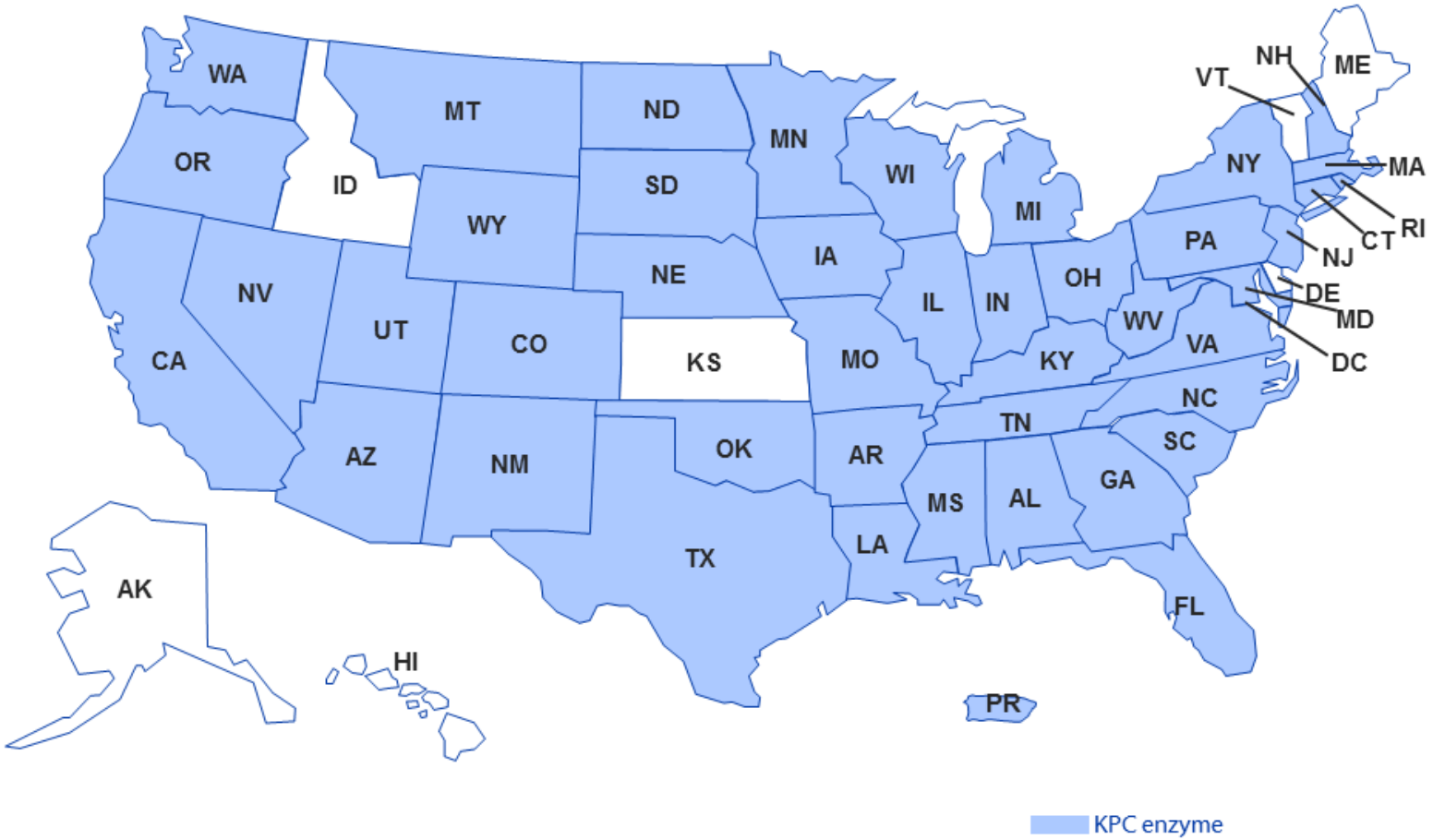
Nordmann et al. *Emerg Inf Dis.* 2011;17:1791-98

Geographic Distribution of KPC Carbapenemases



Nordmann et al. *Emerg Inf Dis*. 2011;17:1791-98

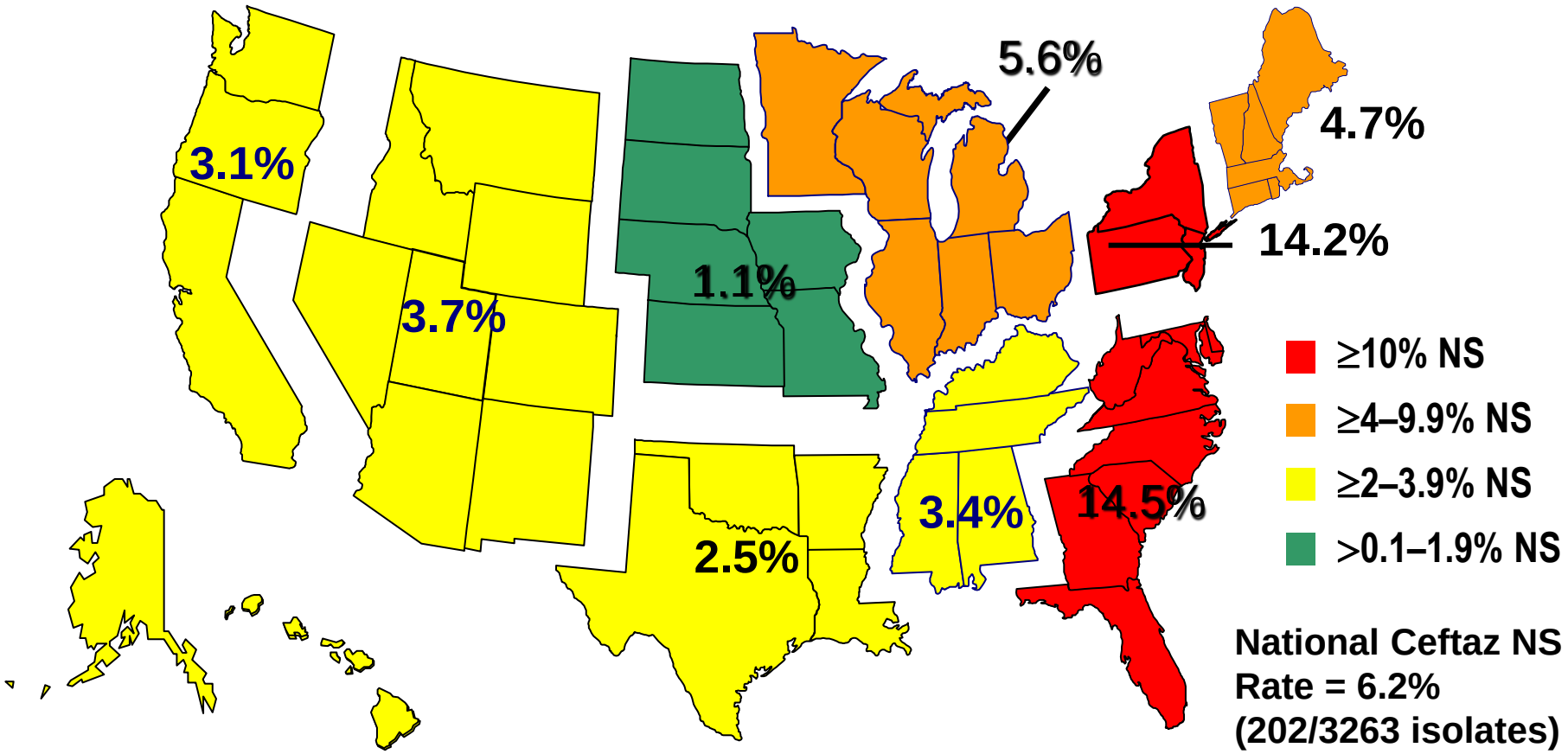
States Reporting KPC Carbapenemases



This map was last updated on September 9, 2013

www.cdc.gov

Regional Ceftazidime-Nonsusceptible Phenotypes of *E coli* and *K pneumoniae*



Yee, Evangelista, Pillar, et al. ASM 2009, abstr/poster A-088.
Ceftazidime-NS defined as an MIC of ≥16 µg/mL; CLSI M100-S19, Jan 2009.

Question #3

What are the revised carbapenem MIC breakpoints (CLSI 2010) and how do the carbapenems differ in activity and by test method?

Determination of MIC Breakpoints
CLSI M23-A3 Guideline (2008)

1. MIC Distribution of Isolates
   - Large number (~500) clinically relevant isolates
   - Varying suscep, geographically diverse areas
2. Pharmacokinetic & dynamic Criteria (PK/PD)
   - Dosing regimens, achievable drug levels in vivo; percent target attainment in population studies
   - Conc-dep killing: AUC/MIC ratio (FQs); 24h area under serum conc time curve to MIC ratio
   - Time-dep killing: T>MIC (β-lactams) ; time that serum drug levels are above MIC
3. Clinical Outcome
   - correlation of MIC to clinical success
   - bacteriologic eradication in treated patients

Enterobacteriaceae: Revised CLSI Antimicrobial Breakpoints (MIC μg/mL)

Agent	CLSI M100-S19 (2009)			CLSI M100-S20 (2010)[a]			
	Susc	Int	Res	Usual Adult Dosage	Susc	Int	Res
Cefazolin	≤8	16	≥32	1g every 8h	≤2	4	≥8
Cefotaxime	≤8	16-32	≥64	1g every 8h	≤1	2	≥4
Ceftriaxone	≤8	16-32	≥64	1g every 24h	≤1	2	≥4
Ceftazidime	≤8	16	≥32	1g every 8h	≤4	8	≥16
Aztreonam	≤8	16	≥32	1g every 8h	≤4	8	≥16
Cefepime[b]	≤8	16	≥32	1g every 12h	≤2[b]	4	≥8
Ertapenem	≤2	4	≥8	1g every 24h	≤0.5[c]	1	≥12
Imipenem	≤4	8	≥16	1g every 8h	≤1	2	≥4
Meropenem	≤4	8	≥16	1g every 8h	≤1	2	≥4
Doripenem	--	--	--	500mg ev 8h	≤1	2	≥4

a: CLSI M100-S20. Table 2A. Jan 2010. b: approved at CLSI AST Jun 2013, for M100-S24 Jan 2014; FEP dosage previously based on 1g every 8h or 2g every 12h. c: CLSI M100-S22. Jan 2012

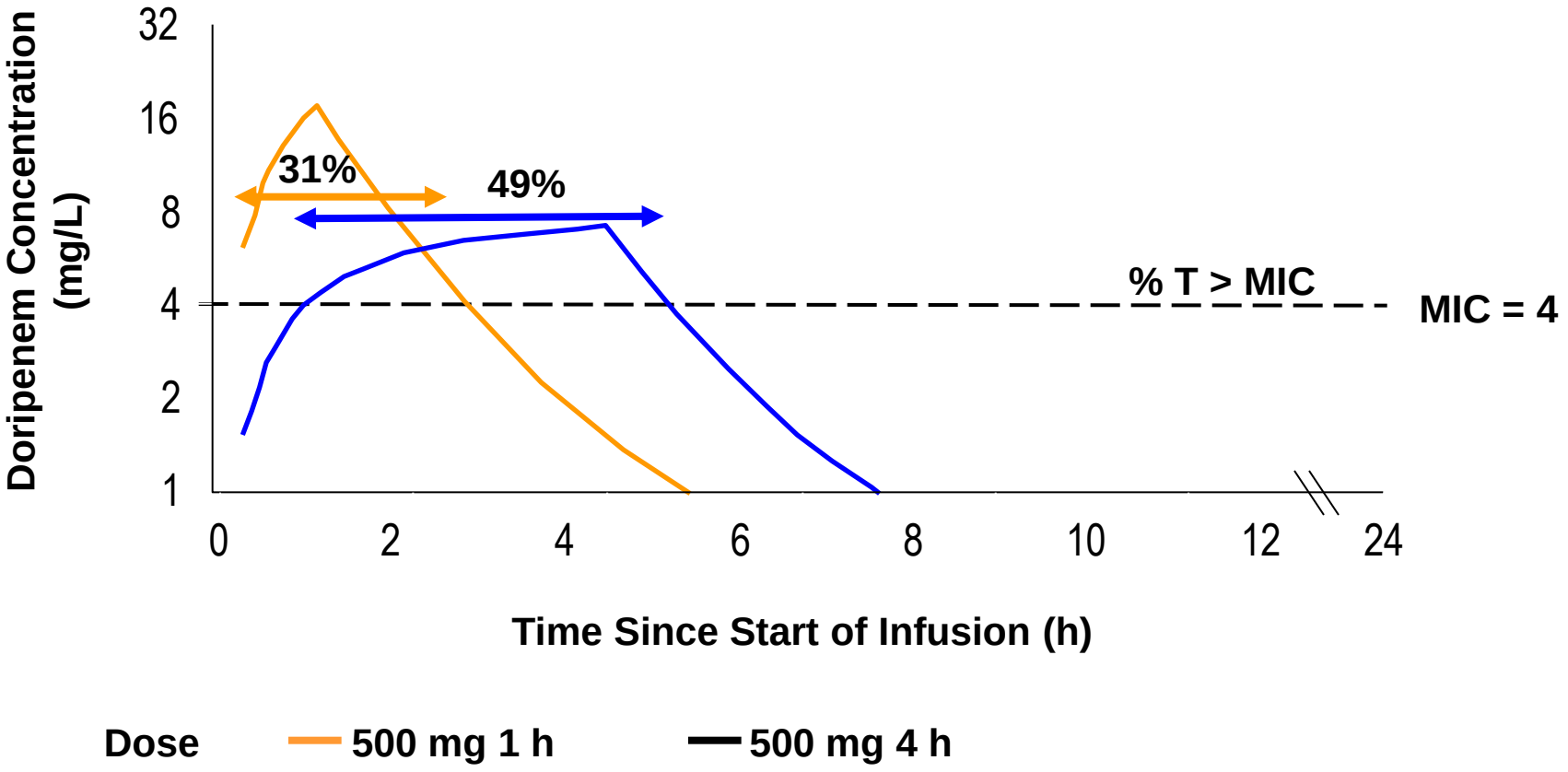
Pharmacodynamic Properties of Beta-Lactams

Antimicrobial Class	Cidal end pt[a] (% of dosing interv)	hrs above MIC for q8h dosing
Cephalosporins	60-70[b]	5.6h
Penicillins	50-60[b]	4.8h
Carbapenems	35-40[b]	3.2h

a: generally considered a 3-log reduction in colony forming units
b: percent of dosing interval required for free drug concentration to be above MIC (%T>MIC)

Drusano et al. *Clin Infect Dis*. 2003;36 (S1):S42-50.

Extended Infusion Regimens
Increase %T > MIC



Bhavnani SM et al. *Antimicrob Agents Chemother*. 2005;49:3944-7.

Question #4

How accurate are commercial antimicrobial susceptibility testing (AST) systems in detecting CRE?

Ability of commercial AST systems to infer carbapenemase production in isolates of Enterobacteriaceae with defined carbapenem-R mechanisms

	Number of Isolates					
	Phoenix		MicroScan NM36		Vitek 2[a]	
Mechanism	Pos	Neg	Pos	Neg	Pos	Neg
KPC (n=8)	8	0	8	0	8	0
MBL (NDM, VIM) (n =16)	16	0	16	0	16	0
OXA-48 (n=11)	11	0	6	5	5	6
ESBL + porin loss (n=10)	10	0	10	0	8	2
AmpC + porin loss (n=6)	6	0	5	1	2	4

Woodford, et al. *J Clin Microbiol.* 2010; 48:2999-3002
a: updated Vitek 2 cards introduced in 2012 and 2013

Comparison of AST systems to detect meropenem resistance in 46 KPC-producing *K pneumoniae*

	Number (%) of Isolates with indicated result		
Testing Method	**Very Major (false S)**	**Major (false R)**	**Minor**
Etest	**1 (2.2)**	**0**	**1 (2.2)**
Vitek 2 (GN28) (discontinued)	**11 (23.9)**	**0**	**18 (39.1)**
Vitek 2 (GN142)	**0**	**0**	**1 (2.2)**
Sensititre	**3 (6.5)**	**0**	**12 (26.1)**
MicroScan	**0**	**0**	**1 (2.2)**

Bulik et al. *J Clin Microbiol*. 2010;48:2402-06

Question #5

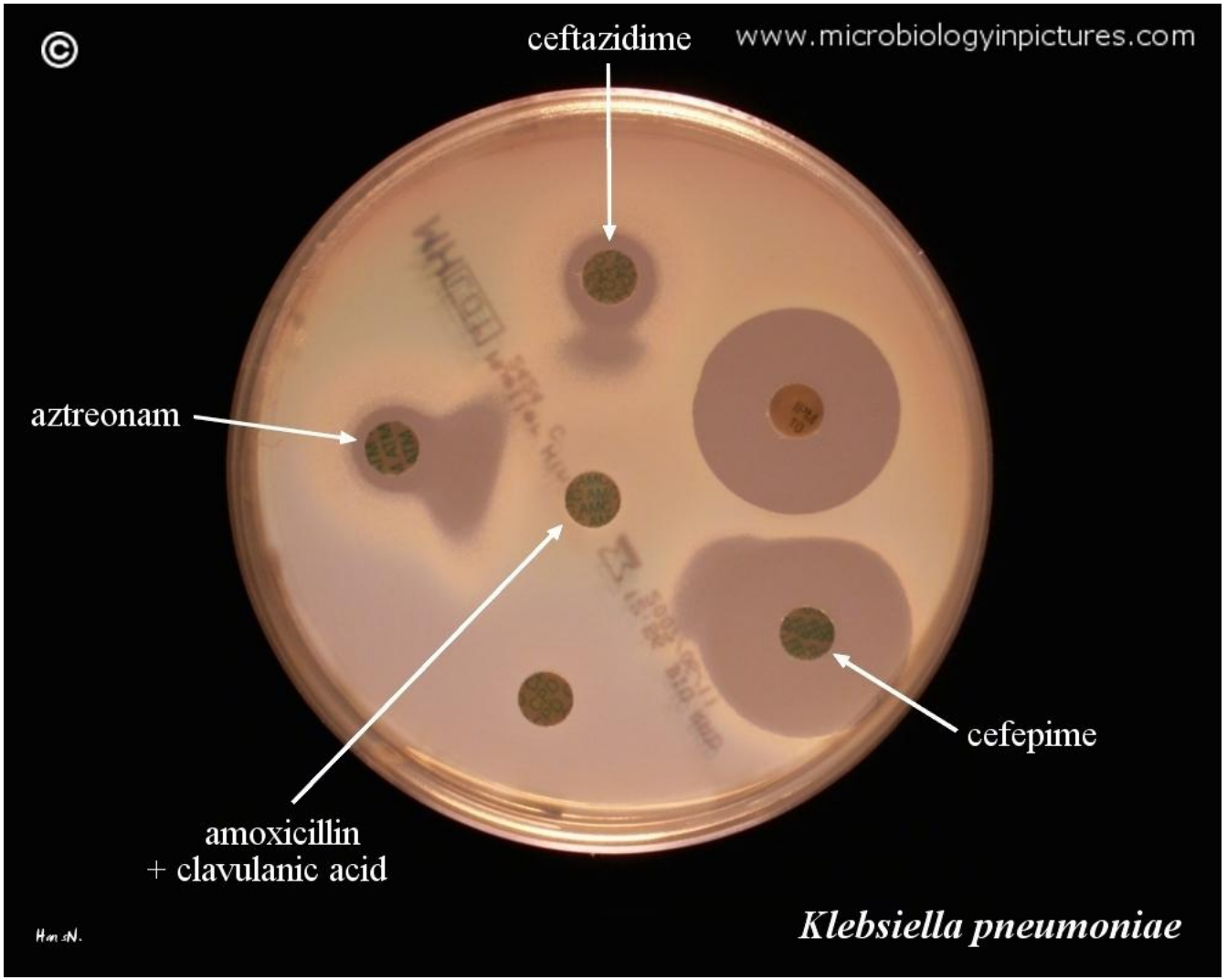
How are ESBL- and AmpC-producers, and CRE detected by phenotypic testing?

Detection of ESBL-Producing Enterobacteriaceae

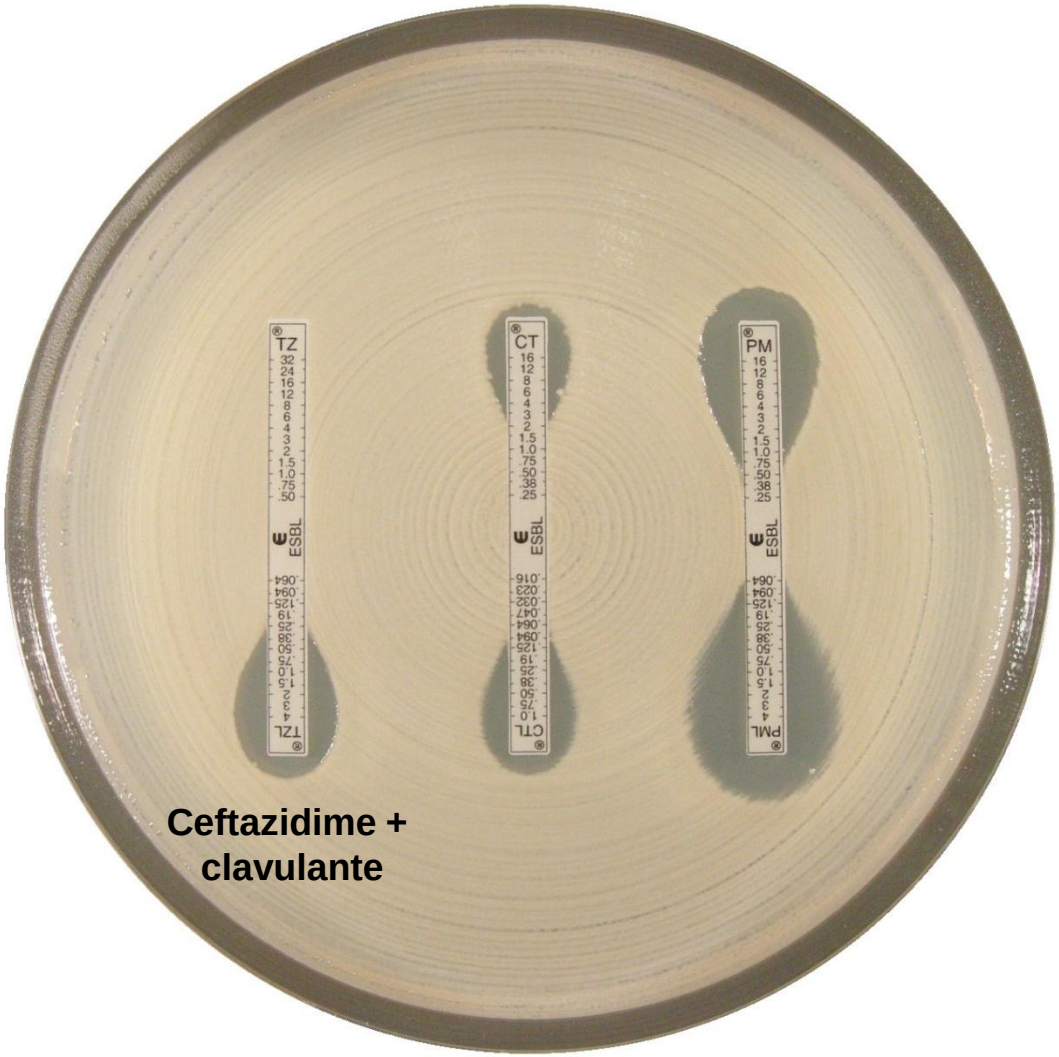
ESBLs (TEM, SHV, CTX-M)

- Inhibited by clavulanate
  - (cefotax + clav = MIC 8-fold lower than cefotax alone)
    (also ceftaz + clav = MIC 8-fold lower than ceftaz alone)
  - Disk test w & w/o clavulanate ≥5mm inh, Double disk syn test
  - Etest TZ and TZ + clav (MIC reduction ≥ 8 fold; RUO in US)
  - Detected by Vitek 2, MicroScan, Phoenix, & Sensititre
- Usually R to cefotaxime/ceftriaxone & ceftazidime
- S to cefoxitin & cefotetan (cephamycins) (AmpC are cefox-R)
- CTX-M are cefotaxime/ceftriaxone-R and ceftaz-S
- Not inhibited by boronic acid in disk (KPC inh)
- Not inhibited by EDTA in disk (MBL inh)
- Not inhibited by cloxacillin when testing carbapenems for AmpC (AmpC inh)

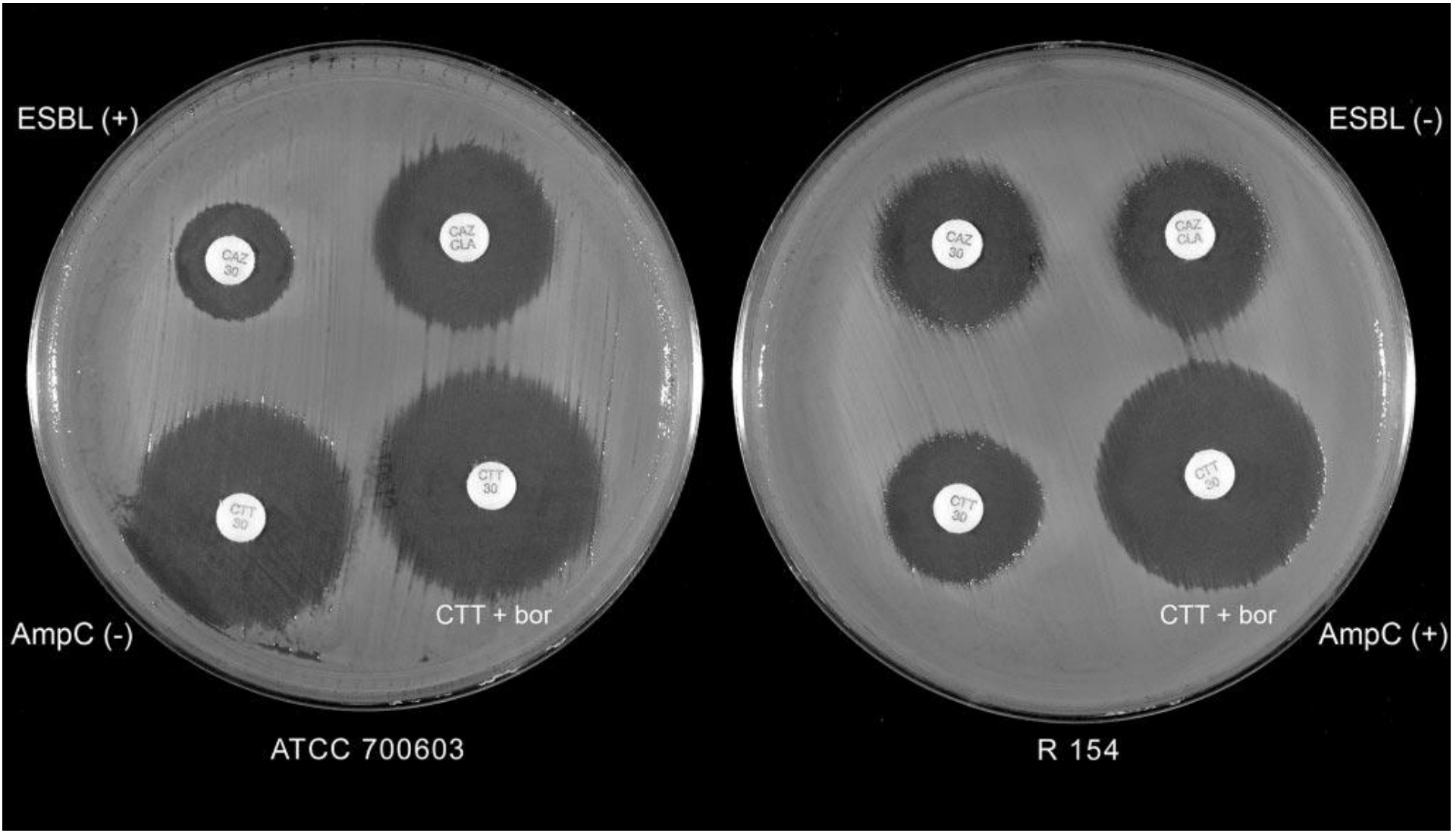
Double Disk Test for ESBL with amox/clav disk in center



Etest for ESBL with clavulanate combo



Confirmatory ESBL test using the ceftazidime and ceftazidime/clavulanic disks



Confirmatory AmpC test using the cefotetan and cefotetan/boronic acid disks. Coudron. *J Clin Microbiol*. 2005;43:4163-67. (inh disks: Rosco Diagnostica, Denmark)

Detection of AmpC-Producing Enterobacteriaceae

AmpC (plasmid-mediated: CMY, FOX, DHA)

- Inhibited by boronic acid
  - cefotetan+ boronic acid ≥5mm inh by disk compared to cefotetan alone
- Inhibited by cloxacillin when testing carbapenems
  - mero + clox ≥5mm inh by disk compared to meropenem alone
  - Etest MP and MP + clox (MIC reduction ≥ 8 fold; RUO in US)
- R to cefotax/ceftriax, ceftazidime, & cefox & cefotetan
- R to cefoxitin & cefotetan (cephamycins)
- Not inhibited by EDTA in disk (MBL inh)
- Not inhibited by clavulanate in disk (ESBL inh)
- May be present w ESBL and mask ESBL clav disk test

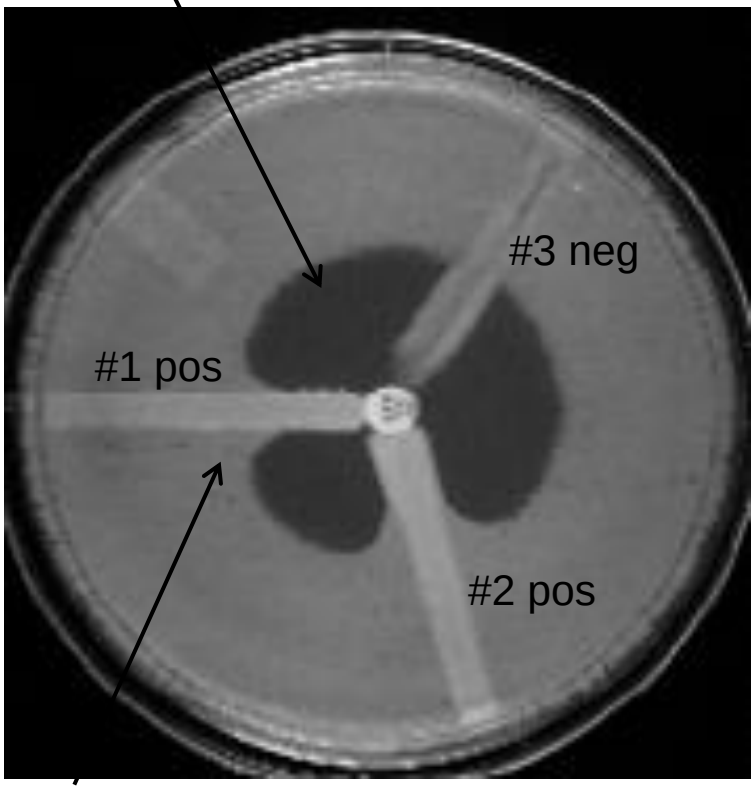
Detection of KPC-Producing Enterobacteriaceae

KPC (KPC-2, KPC-3)

- Strains have a mean of 3.5 other beta-lactamases
  - Usually R to cefotax/ceftriax, ceftazidime, & if carrying AmpC also R to cefox & cefotetan
- Positive by modified Hodge Test (MHT) (carbapenem inactivation test)
- CLSI: MHT not necessary when all carbapenems are I or R with new breakpts: test for Inf Prev & epidemiological purposes
- Inhibited by boronic acid
  - mem + boronic acid ≥5mm inh by disk compared to mem alone
- Not inhibited by cloxacillin when testing carbapenems
- Not inhibited by EDTA in disk (MBL inh)
- Slightly inhibited by clavulanate in disk (ESBL inh)
- KPC-3: common MLST – ST258

KPC-Producing *K pneumoniae*: Modified Hodge Test

Inhibition of *E coli* ATCC 25922 by ertapenem disk



Enhanced growth of test *K pneumoniae* strain due to production of KPC enzyme inhibiting the activity of ertapenem from disk

1. Prepare 1:10 dil of 0.5 McFarland suspension of *E coli* ATCC 25922.
2. Swab onto MHA as for disk diff and place ERT or MEM disk (preferred) on lawn (for isolates w MIC 2-4μg/mL)
3. Streak test isolates (#1-3) from edge of disk outward using sterile loop.
4. Incubate overnight.
5. Look for growth of *E coli* around test streak isolate – indicates KPC-producing strain.

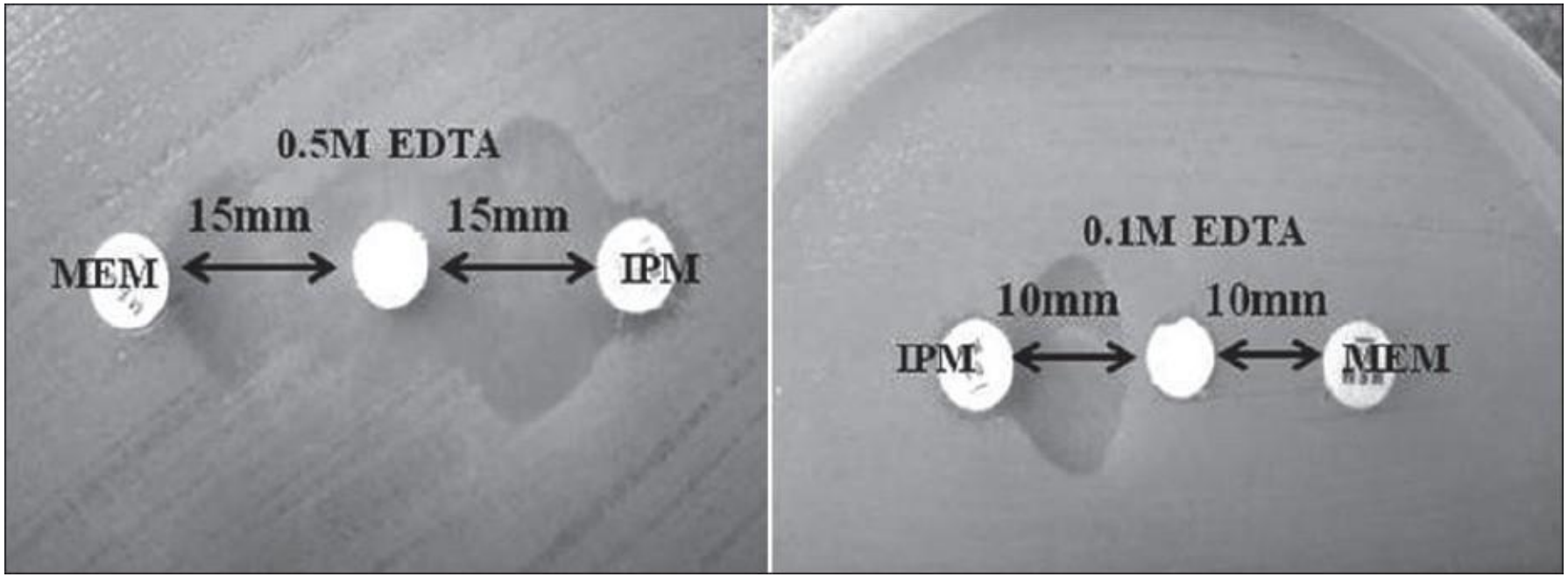
CLSI M100-S22. Jan 2012

Detection of MBL-Producing Enterobacteriaceae

MBL (NDM, VIM, IMP)

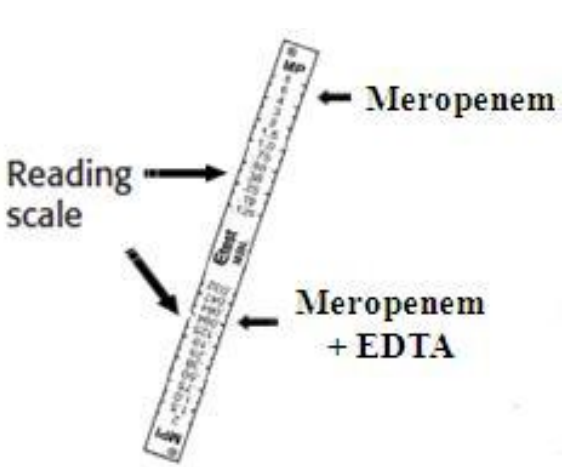
- **Inhibited by EDTA**
  - **mem + EDTA ≥5mm inh by disk compared to mem alone**
  - **Etest MP and MP + EDTA (MIC reduction ≥ 8 fold; RUO in US)**
- **Inhibited by dipicolinic acid (DPA)**
  - **mem + DPA ≥5mm inh by disk compared to mem alone**
- **Strains have other beta-lactamases**
  - **Usually R to cefotax/ceftriax, ceftaz, & if carrying AmpC also R to cefox & cefotetan; aztreonam (AT)-S but usually AT-R due to beta-lactamases other than MBL**
- **Some positive by modified Hodge Test (carbapenem inactivation test; low sensitivity NDM strains, false neg)**
- **Not Inhibited by boronic acid (KPC inh)**
- **Not inhibited by cloxacillin when testing carbapenems**
- **Not inhibited by clavulanate in disk (ESBL inh)**

Detection of MBL: EDTA disk synergy test



Purohit et al. *Ind J Med Microbiol*. 2012;30:456-61

Etest for MBL (NDM, VIM, IMP) with meropenem + EDTA and meropenem



Meropenem + EDTA
(> 8-fold reduction in MIC compared to MP alone

Question #6

What type of screening agars are available for ESBL, AmpC, and and CRE surveillance samples?

Surveillance Testing for MDRO: MRSA, VRE, MRD Gram Neg Pathogens

Surveillance Samples

- **Nasal, axilla, and rectal swabs (pediatric patients)**
  - **37% more pos screen samples w 3 swabs than nasal alone (St Christopher's Hosp for Children, data on file)**

MRSA Screen

- **MRSA Chromogenic agar (BD, Remel/Oxoid, Hardy, CHROM)**
- **CNA (colistin nalidixic acid) agar -> Staph col -> AST**
- **Molecular for mecA -> several MRSA PCR & NAA tests**

VRE Screen

- **VRE Chromogenic agar**
- **CNA (colistin nalidixic acid) agar -> enterococcus col -> vanco screen agar (6μg/mL vanco)**
- **Molecular: Film Array PCR (BioFire) BCID for vanA/B**

Surveillance: MRD Gram Neg Pathogens

MRD-GN Screening agar (for ESBL & AmpC)

- **Vacc agar (Remel/Oxoid): vanco + amphoB + ceftaz + clinda**
- **MAC + ceftaz + cloxacillin (Remel/Oxoid)**
- **ESBL Isolation agar (Remel/Oxoid): cefpodoxime**

Chromogenic Media for MRD-GN Screening

- **CHROMagar ESBL (CHROMagar)**
- **chromID ESBL (bioMerieux)**
- **Brilliance ESBL (Remel/Oxoid)**

Surveillance: CRE Screening Media

Screening media for CRE

- CDC TSB + ertapenem disk: inc overnight -> MAC
- CDC TSB + meropenem disk: inc overnight -> MAC
- MAC + meropenem
- Screening media for ESBL

Chromogenic Media for CRE Screening

- chromID CARBA (bioMerieux)
- Brilliance CRE (Oxoid)
- CHROMagar KPC (CHROMagar)
- SUPERCARBA medium (bioMerieux)

(Gerlich et al.*DMID* 2013; higher sens 96.5% for SUPERCARBA including OXA-48 compared to Brilliance CRE and CHROMagar)

CHROMagar

CHROMagar™ KPC

Typical Appearance of Microrganisms

E.coli CarbapenemR

Dark pink to reddish

Klebsiella, Enterobacter, Citrobacter CarbapenemR

Metallic blue

Non contractual Pictures

CHROMagar, Paris, France

Comparison of Selective Media for CRE

Carba-penemase	n	Brilliance CRE (Oxoid)	chromID CARBA (bioMer)	chromID ESBL (bioMer)	TSB + erta	TSB + mero
KPC	12	11	12	12	9	9
NDM	88	77	85	87	87	85
IMP	9	1	9	9	9	9
VIM	6	5	6	6	6	6
OXA	15	12	13	12	15	14
Total	130	106	125	126	129	126
Other β-lactamases						
ESBL	49	19	11	46	44	35
AmpC	21	9	6	20	19	16
Total	70	28	17	66	63	51
Sens (%)		82	96	97	99	97
Spec (%)		60	76	6	10	27

Wilkinson, et al. *J Clin Microbiol.* 2012;

Surveillance: CRE Screening Molecular

Molecular Methods (require growth of colonies on agar)

- **Commercial PCR: FDA-cleared**
  - **Film Array BCID (blood culture ID) (BioFire) for KPC**
- **Commercial PCR: RUO**
  - **BD MAX PCR (BD) for KPC, NDM, OXA-48**
  - **Check-MDR Carba (Check-Points, The Netherlands)**
    for KPC, NDM, VIM, IMP, OXA-48
    equipt req'd: DNA extraction method (e.g. EasyMag), thermocycler, PCR plate spinner
- **PCR, Home Brew: RUO for *bla*$_{KPC}$ PCR**
- **Mass Spectrometry (RUO): (growth on agar)**
  - **MALDI (matrix-assisted laser desorption ionization)**
  - **Vitek MS: detect peak shift in substrate (spt) by MS after growth in carbapenem solution (3h growth)**

Question #7

What are the recognizable MDR-GN phenotypes and what are some suggestions for reporting CRE results?

MRD-GN and CRE Reports

Positive for ESBL

- Phenotype: R to 3rd gen ceph, S to cefoxitin, inh by clavulanate
- **"The isolate produces an extended spectrum beta lactamase (ESBL) and is resistant to all penicillins, cephalosporins, and aztreonam." (CLSI M100-S23, Jan 2013)**

Positive for AmpC (w or w/o porin loss or efflux)

- Phenotype: R to 3rd gen ceph, R to cefoxitin, inh by cloxacillin
- **"Testing for ESBL not indicated due to other mechanisms of high level 3rd generation cephalosporin resistance. The isolate is resistant to all penicillins, cephalosporins, and aztreonam."**

Positive for KPC Carbapenemase

- Phenotype: R to 3rd gen ceph, usu R to cefoxitin, pos by MHT
- **"The isolate demonstrates KPC carbapenemase production, which confers resistance to all penicillins, cephalosporins, aztreonam, and carbapenems. The clinical efficacy of carbapenems to treat infections due to these isolates has not been established."**

MRD-GN and CRE Reports

Positive for MBL Carbapenemase (NDM, VIM, IMP – less frequently seen in US than KPC)

- **Phenotype: R to 3rd gen ceph, can be pos by MHT, inhibited by EDTA**
- **aztreonam (AT)-S but usually AT-R due to beta-lactamases other than MBL (MIC results determine susceptibility to AT)**
- **"The isolate demonstrates MBL carbapenemase production, which confers resistance to all penicillins, cephalosporins, and carbapenems. "**

Positive for OXA Carbapenemase (less frequently seen in US than KPC or NDM among Enterobacteriaceae)

- **Phenotype: R to carbapenems (MIC results determine susceptibility to 3rd gen ceph and aztreonam)**

CLSI Notes for MHT and CRE Detection
(CLSI M100-S23, Jan 2013)

1. **The imipenem disk performs poorly as a screen for carbapenemases in the MHT. Use ertapenem or meropenem disks.**
2. **The screening and confirmatory test recommendations (MHT) were largely derived following testing of US isolates of Enterobacteriaceae and provide high sensitivity (>90%) and specificity (>90%) in detecting KPC-type carbapenemases in these isolates.**
3. **Not all carbapenemase-producing isolates of Enterobacteriaceae are MHT positive (NDM-producing isolates have low sensitivity).**
4. ***Proteus* spp, *Providencia* spp, and *Morganella* spp. may have elevated MICs to imipenem by mechanisms other than production of carbapenemases (Omp loss); thus the usefulness of the imipenem MIC screen test for the detection of carbapenemases is not established for these 3 genera.**
5. **MHT-positive results (false pos) may be encountered in isolates with carbapenem resistance mechanisms other than carbapenemase production.**