



ESBLs and KPCs: Impact of Revised CLSI Breakpoints on testing and Reporting Algorithms

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Extended-Spectrum β -lactamases among *Enterobacteriaceae*

- Derivatives of TEM-1, TEM-2 and SHV-1 β -lactamases (1 - 4 amino acid substitutions)
 - Most commonly observed with:
 - *Escherichia coli*
 - *Klebsiella pneumoniae*
- Characterized by substrate profile, isoelectric point and gene/amino acid sequence

[<http://www.lahey.org/studies/webt.htm>]

Phenotype of ESBL-producing *Enterobacteriaceae*

- Elevated MICs to penicillins, cephalosporins (including advanced generation agents) and aztreonam
 - MIC increases can be extremely modest
 - Part of rationale for changes in breakpoints
- Susceptible to cephamycins and carbapenems
- Usually inhibited by clavulanate (IRTs)

ESBL Testing

- With publication of revised breakpoints by SAST in 1/10, ESBL testing were no longer necessary except for epidemiologic purposes
- New breakpoints captured organisms for which interpretations would otherwise have needed to have been changed using prior breakpoints (when ESBL production was confirmed)

CLSI *Enterobacteriaceae* Interpretive Criteria

MIC Breakpoints Published by CLSI SAST

| <u>Drug</u> | (μg/mL) | | |
|-------------|--------------------|---------------------|------------------|
| | <u>Susceptible</u> | <u>Intermediate</u> | <u>Resistant</u> |
| Cefazolin | ≤ 2 (≤ 8)** | 4 (16) | ≥8(≥32) |
| Cefotaxime | ≤ 1 (≤ 8) | 2 (16-32) | ≥4 (≥64) |
| Ceftizoxime | ≤ 1 (≤ 8) | 2 (16-32) | ≥4 (≥64) |
| Ceftriaxone | ≤ 1 (≤ 8) | 2 (16-32) | ≥4 (≥64) |
| Ceftazidime | ≤ 4 (≤ 8) | 8 (16) | ≥16 (≥32) |
| Aztreonam | ≤ 4 (≤ 8) | 8 (16) | ≥16 (≥32) |

Disc Breakpoints Published by CLSI

| <u>Drug</u> | (mm) | | |
|-------------|--------------------|---------------------|------------------|
| | <u>Susceptible</u> | <u>Intermediate</u> | <u>Resistant</u> |
| Cefazolin | ≥23 (≥18) | 20-22 (15-17) | ≤19 (≤14) |
| Cefotaxime | ≥26 (≥23) | 23-25 (15-22) | ≤22 (≤14) |
| Ceftizoxime | ≥25 (≥20) | 22-24 (15-19) | ≤21 (≤14) |
| Ceftriaxone | ≥23 (≥21) | 20-22 (14-20) | ≤19 (≤13) |
| Ceftazidime | ≥21 (≥18) | 18-20 (15-17) | ≤17 (≤14) |
| Aztreonam | ≥21 (≥22) | 18-20 (16-21) | ≤17 (≤15) |

** Previous breakpoints are in parentheses

Obstacles to Changing Breakpoints and Rationale for Continuing to Test

- ESBLs
 - Inoculum effect – *in vitro*
 - Reports of clinical failures
 - Impact of increased inocula in animal models
 - Differences in results when testing is performed by various methods
 - Reproducibility of MIC testing
 - FDA versus CLSI
 - Infection control needs

Obstacles to Changing Breakpoints and Rationale for Continuing to Test

- Carbapenemase producing enterics
 - Lack of applicable dilutions on automated systems
 - Inoculum effects
 - FDA versus CLSI and manufacturer's requirements
 - Infection control / epidemiology needs

ESBLs – Original Rationale for Testing

- MICs of many β -lactams for ESBL producing organisms fell within susceptible range
- Limited numbers of reports of clinical failures among patients with infections caused by ESBL-producing organisms when treated with β -lactams to which the organisms tested “S” *in vitro*¹

ESBLs – Original Rationale for Testing

- *In vitro* hydrolysis of β -lactams by ESBLs varies significantly based on inoculum size
- General lack of validated PK/PD data on older β -lactam antimicrobial agents
- As carbapenems were uniformly active at the time versus the Enterobacteriaceae, “overcalling” resistance to the cephalosporins and aztreonam still left effective antibiotic options
- Original recommendation to report all ESBL-producing organisms as resistant to all cephalosporins and penicillins was intended to be a short term solution to address a newly recognized resistance mechanism

Basis for Changes in Interpretive Criteria

- Numerous additional mechanisms of resistance have now been identified (e.g., new ESBLs including CTX-M types, plasmid-mediated ampC-like enzymes, KPCs, etc.)
- Increasing prevalence of organisms harboring multiple resistance mechanisms that render confirmatory (clavulanate effect) testing non-interpretable (i.e., ampC and ESBL)

Monte Carlo Simulations

- PD parameters such as the rate of bactericidal activity with increasing drug concentrations, the post-antibiotic effect, sub-MIC effects, post-antibiotic leukocyte enhancement, and the 1st exposure effect more accurately describe the time course of antimicrobial activity *in vivo* than the MIC (or MBC)
- Inoculum effect (*in vivo*)
- Monte Carlo simulations do not work in clinically important sub-MIC ranges
- “Inflated PK variance”?

ESBLs

Basis for Changes in Interpretive Criteria

- Carbapenems are no longer routinely active against all isolates of Enterobacteriaceae (KPCs, metallo- β -lactamases (i.e., NDM-1))
- May be driving carbapenem resistance by reporting ESBL-producing organisms as resistant to all other β -lactams
- CLSI revised cephalosporin and aztreonam breakpoints better represent the clinical effects these compounds have with currently recommended antibiotic dosage regimens when treating infections caused by contemporary bacterial isolates

ESBLs

Basis for Changes in Interpretive Criteria

- Supported by improved understanding of the pharmacokinetic and pharmacodynamic (PK/PD) determinants of efficacy with β -lactam agents (Monte Carlo simulations)
- New interpretive criteria obviate the need for laboratories to perform ESBL screening and confirmatory tests for directing treatment decisions (although they may still be needed for infection control and/or epidemiologic purposes)

Obstacles to Changing Breakpoints and Rationale for Continuing to Test for ESBL Production

- Few (rare) reports have been cited of patients infected with ESBL-producing organisms testing susceptible using new breakpoints, but failing therapy
- However, no randomized controlled trials have ever been performed that evaluated the use of various comparator antibiotics in the treatment of serious infections due to ESBL-producing organisms
- Unlikely that such studies will ever be performed
- Existing data are only from retrospective studies
- In previously cited Paterson study, only 3 of 11 patients with serious infections failed Rx when the β -lactam MIC was $\leq 1 \mu\text{g/mL}$ (statistical significance; attributable?; background failure rate?)

Concerns for Clinical Failure

- Karas et al reported treatment failure in a patient with sepsis and pneumonia caused by an ESBL-positive *Klebsiella pneumoniae* treated with cefotaxime based on the lab report of “S” by disk diffusion testing
- After 2 days of treatment, the patient’s condition deteriorated and an Etest was performed; cefotaxime MIC of 0.75 µg/mL (below the new CSLI susceptible breakpoint)
- Therapy with cefotaxime was stopped
- Patient’s Rx was switched to ciprofloxacin

Concerns for Clinical Failure

- Clinical improvement noted the next day
- Authors noted that removal of the central venous catheter did not contribute to resolution of the infection and that the patient only improved when cefotaxime was replaced with ciprofloxacin
- Authors postulated that if an infectious site in a patient has a high concentration of ESBL producing organisms (i.e.; $>10^7$ CFU/mL), cephalosporin failure is likely

Concerns for Clinical Failure

- Because of recognized inoculum effect with ESBL-producing organisms, concerns have been raised that inoculum used in broth microdilution tests (5×10^5 CFU/mL) may be too low, potentially diluting out resistant subpopulations (thereby generating occasional false susceptible results)
- Thomson et al reported that when a known ESBL (SHV-3) producing strain of *Citrobacter freundii* was tested by broth microdilution using an inoculum of 5×10^5 CFU/mL, the MICs were: cefotaxime 2 μ g/mL, ceftazidime 1 μ g/mL, aztreonam 0.5 μ g/mL and cefepime 0.5 μ g/mL (all Susceptible)

Inoculum Size Concerns

- When the testing was repeated using an inoculum size of 5×10^7 CFU/mL, the MICs were: cefotaxime 256 $\mu\text{g/mL}$, ceftazidime 32 $\mu\text{g/mL}$, aztreonam 32 $\mu\text{g/mL}$, and cefepime >1024 $\mu\text{g/mL}$ (all Resistant)
- But, *C. freundii* also typically harbors an inducible ampC gene
- Paterson et al also reported that a patient died after receiving cephalosporin monotherapy based on a laboratory report of “S”; the *in vitro* MICs for the isolate increased when a 10-fold increase in inoculum was used (the ceftriaxone MIC \uparrow from 8 to >256 $\mu\text{g/mL}$ and the cefepime MIC \uparrow from 0.5 to 8 $\mu\text{g/mL}$)

Effect of Inoculum Size in Animal Models

- Using a rat intra-abdominal abscess model, Rice et al showed that extended spectrum cephalosporins may be less effective in treating serious infections due to ESBL producing bacteria than standard susceptibility tests would imply
- Their *in vitro* studies showed that the activity of these agents against an ESBL producing strain of *K. pneumoniae* was highly inoculum dependent
- They recommended avoiding use of extended spectrum cephalosporins as single agents when treating serious infections with ESBL-producing organisms

Rice LB, Yao JD, Klimm K, Eliopoulos GM, Moellering RC Jr. Efficacy of different beta-lactams against an extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* strain in the rat intra-abdominal abscess model. *Antimicrob Agents Chemother.* 1991 Jun;35(6):1243-4

Obstacles to Changing Breakpoints and Rationale for Continuing to Test for ESBL Production

- Host response?
- Various susceptibility testing methods have been shown to generate different results

Concerns Regarding Fact That Different Susceptibility Testing Methods Generate Varying Results

- In the CAP survey D-C 2007 D-19, laboratories were asked to perform susceptibility testing on a *Citrobacter freundii* strain containing a PER-1 ESBL with a reference cefepime MIC of 16 µg/mL (Intermediate)
- 15% of 311 MicroScan users reported cefepime susceptible, 97% of 244 Vitek Legacy users reported cefepime susceptible, 86% of 230 Vitek 2 users reported cefepime susceptible, and 34% of 74 disk diffusion users reported cefepime susceptible

Concerns Regarding Fact That Different Susceptibility Testing Methods Generate Varying Results

These proficiency survey results demonstrate that the same isolate tested in various laboratories using different methods can lead to widely disparate results, thus calling into question the reliance on standard susceptibility testing methods alone as the sole criteria for determining antimicrobial susceptibility

Concerns Regarding the Reproducibility of MIC Results

- The published CLSI QC Table entitled: “Acceptable limits of QC strains used to monitor accuracy of standard susceptibility tests” allows between a 3 and 8 two-fold dilution range when performing QC testing with a defined control organism *E. coli* ATCC 25922
- Results within this 3 to 8 dilution range are considered to be “in control” when using standardized susceptibility test systems

Concerns Regarding the Reproducibility of MIC Results

- Possible that an ESBL producer with a cefotaxime MIC of 1 $\mu\text{g}/\text{mL}$ (probably responsive to cefotaxime *in vivo*) cannot be reliably distinguished from one with an MIC of 4 $\mu\text{g}/\text{mL}$ (possibly non-responsive)
- Is it therefore “safer” to take a cautious approach and screen for ESBLs and if found, report the isolate as resistant to all β -lactams except the carbapenems?

Concerns Regarding Different Interpretations for Various β -lactams

- EUCAST (European Committee for Antimicrobial Susceptibility Testing) epidemiologic cut-off values for the wild type strains of Enterobacteriaceae are $\leq 0.5 \mu\text{g/mL}$ for most species with the cephalosporin antibiotics
- Indicates that strains with extended spectrum cephalosporin MICs $>1 \mu\text{g/mL}$ have acquired some mechanism of antibiotic resistance
- EUCAST chose to lower the susceptible breakpoints to $\leq 1 \mu\text{g/mL}$ for ceftazidime, aztreonam, and cefepime so that organisms possessing ESBLs would test non-susceptible to these drugs as well

Elimination of ESBL Confirmatory Testing

- Cost and labor savings;
 - \$9.00 for ESBL confirmatory Etest strips
 - \approx \$1.00 for Mueller Hinton Plate
 - Cost of swab, saline, tube, etc.
 - Technologist time
- Modest estimate of \$20/isolate tested
- > 400 ESBL producing strains at NYP/WCMC last year
- Minimum of \$8,000 cost savings/year

Proposed Alternative Approach to ESBL Testing

- Use cefotaxime (CTAX) or ceftriaxone (CTRX) as a surrogate marker for ESBL production
- 99% of confirmed ESBL producers in 2009 at NY Presbyterian Hospital/Weill Cornell Medical Center were ceftriaxone-resistant
- If an enteric isolate is resistant to CTRX or CTAX (other than a SPICE/SPACE organism), enter a comment in the report such as the following: “This multi-drug resistant organism may not respond optimally to β -lactams (other than the carbapenems) or the β -lactam/ β -lactamase inhibitor combinations
- Also serves as a flag for Infection Control purposes

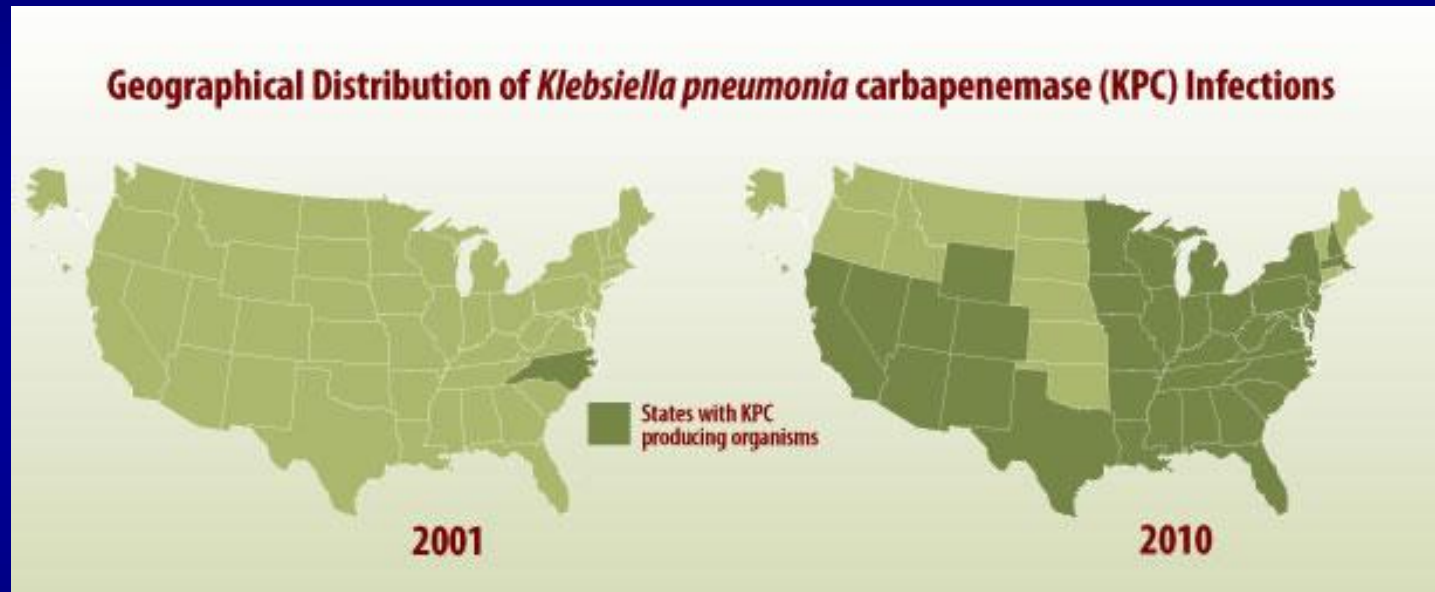
Carbapenemases

- Class A: KPC (1 →11), SME, IMI, NMC
→ serine at the active site
- Class B: IMP-1 →30 , VIM-1 →30, GIM, SPM, SIM, IND-1 →7, NDM-1 →6
→ Zn²⁺-dependent metallo-enzyme
- Class C: N/A
- Class D: OXA family (1 →224+)

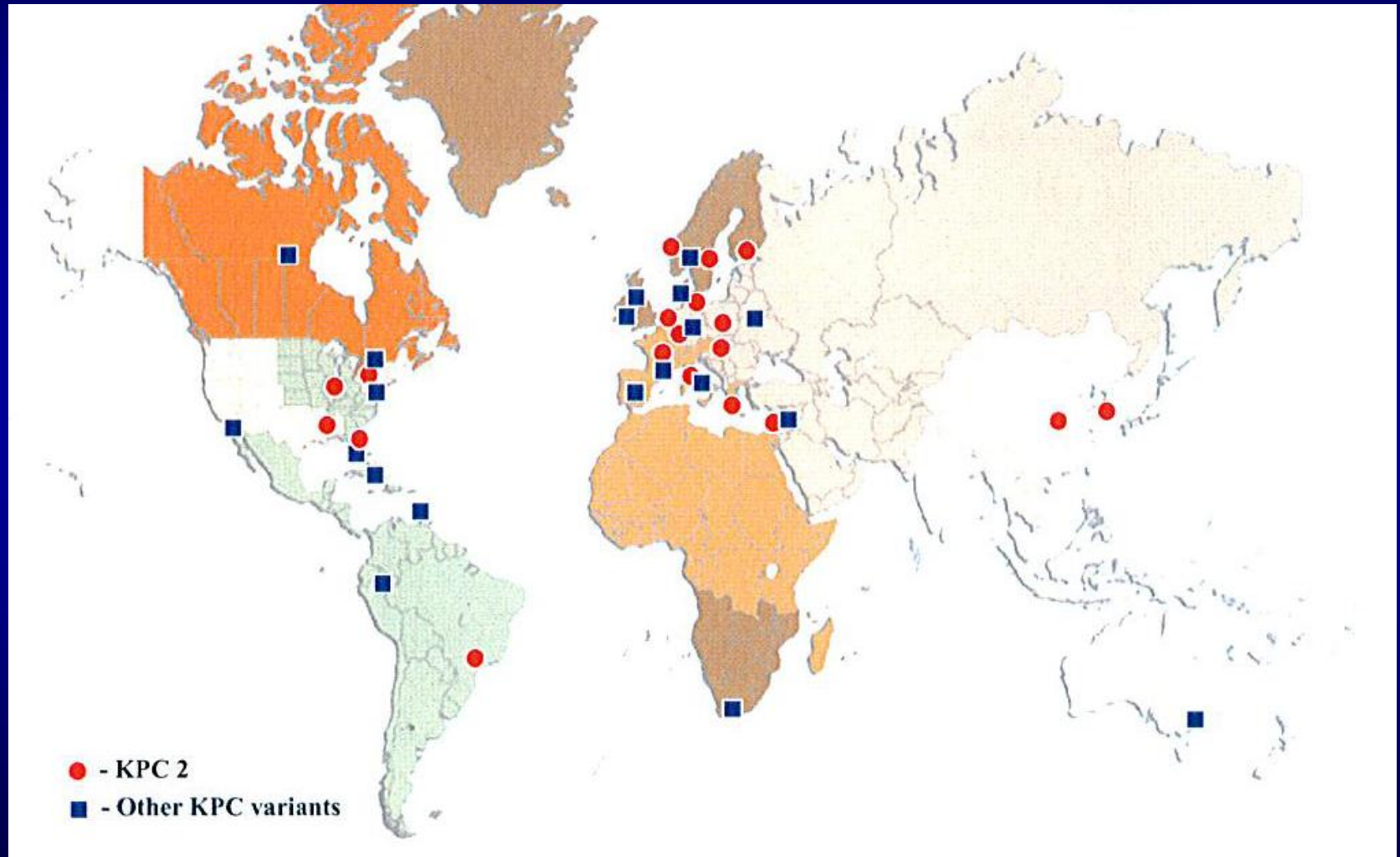
Rationale for Implementation of CLSI Enterobacteriaceae Breakpoint Changes

- Carbapenemase producing enterics
 - PK/PD issues
 - Clinical outcome data
 - Elimination of modified Hodge testing saves valuable laboratory resources
 - Limited treatment options – alternative dosing approaches

KPC-related Carbapenem-Resistant Enterobacteriaceae



Worldwide Distribution of KPC



Susceptibility Testing

- MICs for imipenem may be close to intermediate breakpoint
- Resultantly, interpretations vary from isolate to isolate and from test to test with a given patient
- 15 known CRKP isolates from Brooklyn¹⁰
- Tested for carbapenem resistance using various automated systems
- All systems failed to detect resistance in 1 and up to 10 (67%) of the isolates
- Discordance between meropenem and imipenem susceptibility¹¹

¹⁰Tenover et al. *Emerging Infectious Diseases*. 2006;12(8):1209-13. ¹¹Bratu et al. *Antimicrob Agents Chemother*. 2005;49(7):3018-20

Susceptibility Testing

Frequency of Very Major, Major, and Minor Errors

Testing Method

Number (%) of Isolates with Indicated Result

Very Major

Major

Minor

2010 CLSI Meropenem Interpretive Criteria

| | | | |
|------------|-----------|-------|-----------|
| Etest | 1 (2.2) | 0 (0) | 1 (2.2) |
| Vitek 2 | 11 (23.9) | 0 (0) | 18 (39.1) |
| Sensititre | 3 (6.5) | 0 (0) | 12 (26.1) |
| Microscan | 0 (0) | 0 (0) | 1 (2.2) |

FDA Meropenem Interpretive Criteria

| | | | |
|------------|-----------|-------|-----------|
| Etest | 1 (2.2) | 0 (0) | 7 (15.2) |
| Vitek 2 | 27 (58.7) | 0 (0) | 8 (17.4) |
| Sensititre | 27 (58.7) | 0 (0) | 12 (26.1) |
| Microscan | 0 (0) | 0 (0) | 2 (4.3) |

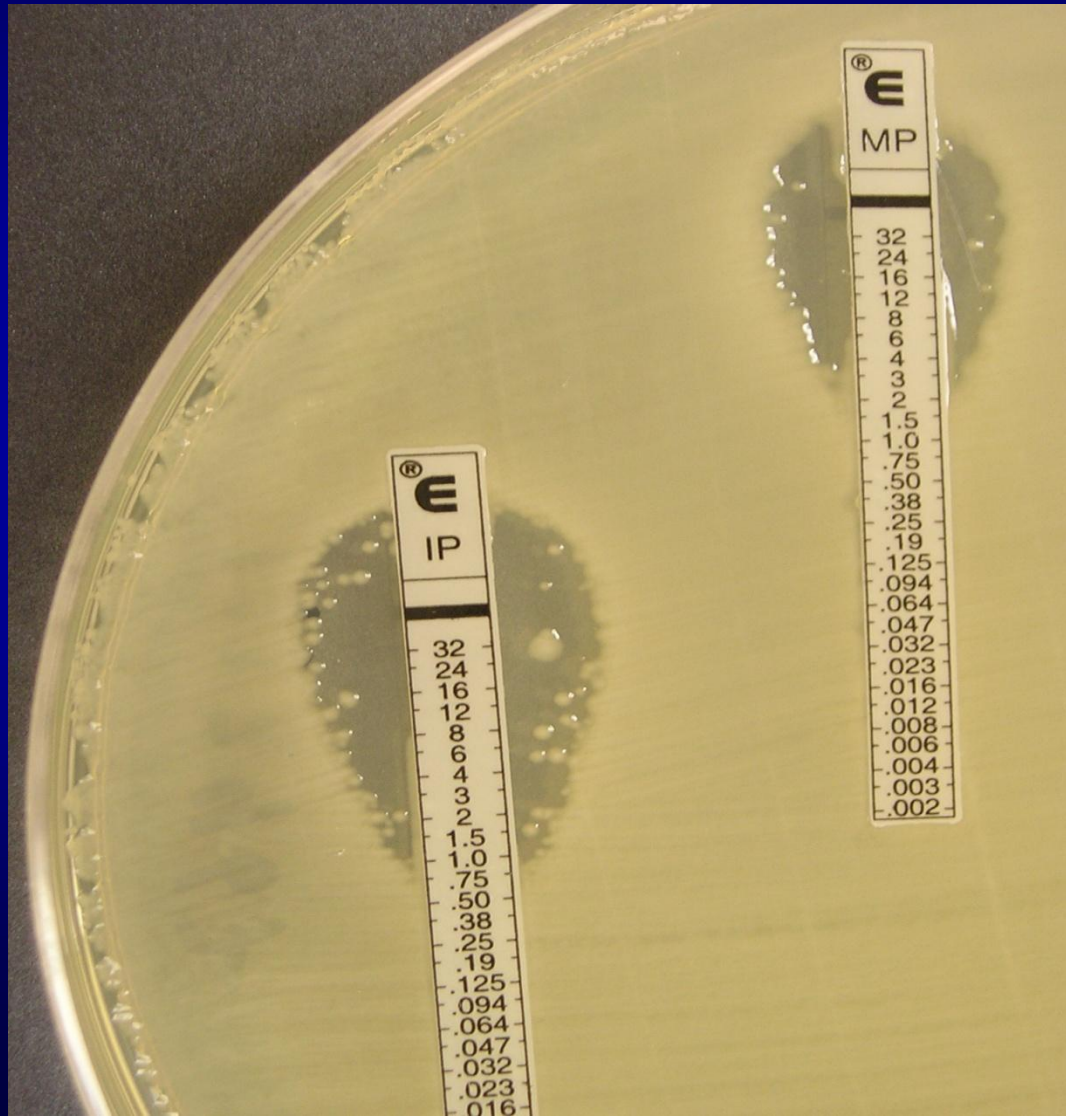
MSMC Microscan Results for Carbapenem Resistant *Klebsiella pneumoniae* (n = 531)

| | <u>S</u> | (%) | <u>R</u> |
|--------------|----------|------|----------|
| Ertapenem | 0 | 0.6 | 99.4 |
| Meropenem | 10.5 | 7.5 | 82 |
| Imipenem | 11.9 | 19.2 | 68.9 |
| Cefepime | 3.8 | 12.4 | 83.8 |
| Tetracycline | 79.1 | 9.8 | 11.1 |
| Amikacin | 25.6 | 46.3 | 28.1 |
| Gentamicin | 55.7 | 15.6 | 28.6 |

Detection of KPC-Mediated Resistance

- **Originally proposed solutions:**
 - Increasing inoculum size to 10^7 (significant inoculum effect)
 - Using ertapenem as a screening reagent
 - E-test or disk (many intermediate) testing for confirmation
 - Lowering meropenem and imipenem breakpoints
 - Perform a modified Hodge test
 - Significant inoculum effect renders detection problematic; ertapenem may be the best “reagent” to detect them

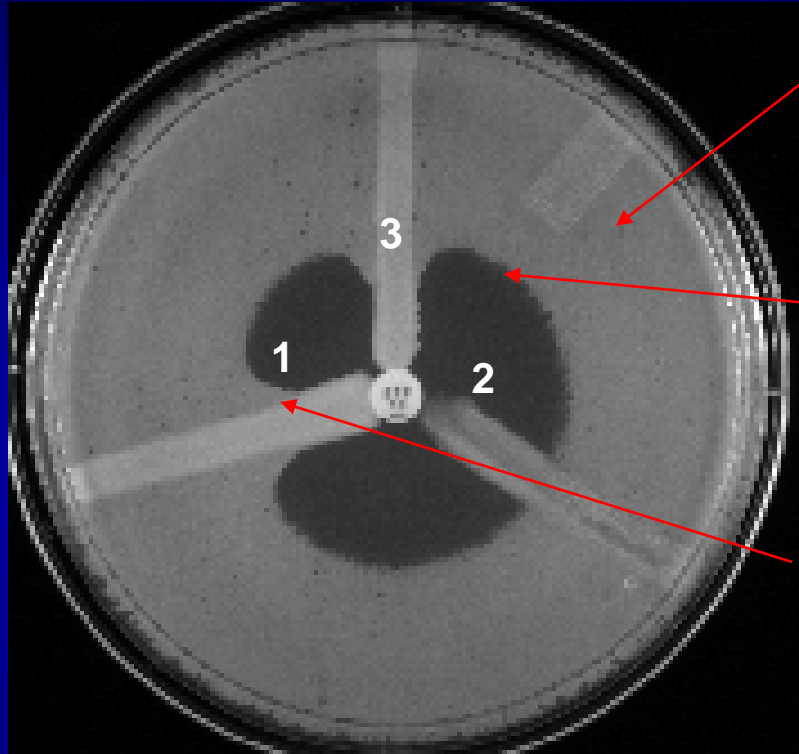
Detection of KPC-mediated Resistance



false



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

New CLSI Carbapenem Breakpoints

MIC Breakpoints Recently Approved by CLSI SAST ($\mu\text{g/mL}$)

| <u>Drug</u> | <u>Susceptible</u> | <u>Intermediate</u> | <u>Resistant</u> |
|-------------|--------------------|---------------------|------------------|
| Imipenem | ≤ 1 (4) | 2 (8) | ≥ 4 (16) |
| Meropenem | ≤ 1 (4) | 2 (8) | ≥ 4 (16) |
| Doripenem | ≤ 1 (NA) | 2 (NA) | ≥ 4 (NA) |
| Ertapenem | ≤ 0.25 (2) | 0.5 (4) | ≥ 1 (8) |

Disc Breakpoints Recently Approved by CLSI SAST (mm)

| <u>Drug</u> | <u>Susceptible</u> | <u>Intermediate</u> | <u>Resistant</u> |
|-------------|-------------------------|---------------------|-------------------------|
| Imipenem | ≥ 23 (≥ 16) | 20-22 (14-15) | ≤ 19 (≤ 13) |
| Meropenem | ≥ 23 (≥ 16) | 20-22 (14-15) | ≤ 19 (≤ 13) |
| Doripenem | ≥ 22 (NA) | 20-21 (NA) | ≤ 19 (NA) |
| Ertapenem | ≥ 23 (≥ 19) | 20-22 (16-18) | ≤ 19 (≤ 15) |

CARBAPENEMS - CLSI

- **New (revised) interpretive criteria for carbapenems were established following evaluation of:**
 - **The pharmacokinetic-pharmacodynamic properties**
 - **Monte Carlo simulations**
 - **Limited clinical data, and**
 - **MIC distributions**

CARBAPENEMS - CLSI

- Due to lack of data from controlled clinical trials there remains some uncertainty whether or not the carbapenems might be effective in the treatment of infections due to *Enterobacteriaceae* with carbapenem MICs or zone diameters that fall within the intermediate range or at the breakpoint for resistance

CARBAPENEMS - CLSI

- **In the setting of limited treatment options, clinicians managing infections due to these isolates may wish to consider maximum approved dosage regimens and/or prolonged intravenous infusions of carbapenems as described in the medical literature**
- **Each laboratory should develop a mechanism for informing clinicians about such circumstances in a timely manner. This might include a telephone call and/or a comment appended to the laboratory report. Consultation with an Infectious Disease specialist is recommended.**

CARBAPENEMS

- **NOTE:** Imipenem MICs for *Proteus* spp, *Providencia* spp, and *Morganella morganii* tend to be higher (e.g., MICs in the intermediate and at the breakpoint of resistance) than those with meropenem or doripenem MICs. These isolates can be imipenem resistant by mechanisms other than production of carbapenemases.
- Until laboratories implement the new interpretive criteria, the modified Hodge test (MHT) should be performed as described in the updated Supplemental Table 2A-S2. After implementation of the new interpretive criteria, the MHT need not be performed other than for epidemiologic or infection control purposes.

Mechanisms of Carbapenem Resistance

- In U.S., Harboring KPC enzyme most frequent etiology
 - Cross-resistance with fluoroquinolones and aminoglycosides
- Hyperproduction of ampC or CTX-M β -lactamases along with an outer membrane porin mutation⁹ ; OMP K37?

⁸*Reviews on Medical Micro.* 2004;15:63-72

⁹*Antimicrob Agents Chemother.* 1997;41(3):563-9

Conclusions

- Microbiology laboratories should consider all of the implications of breakpoint changes before implementing them, but should move forward with the process
- Communication with applicable medical staff members is essential during this somewhat confusing endeavor