Antimicrobial Susceptibility Testing: Perspective on Current Reference Methods

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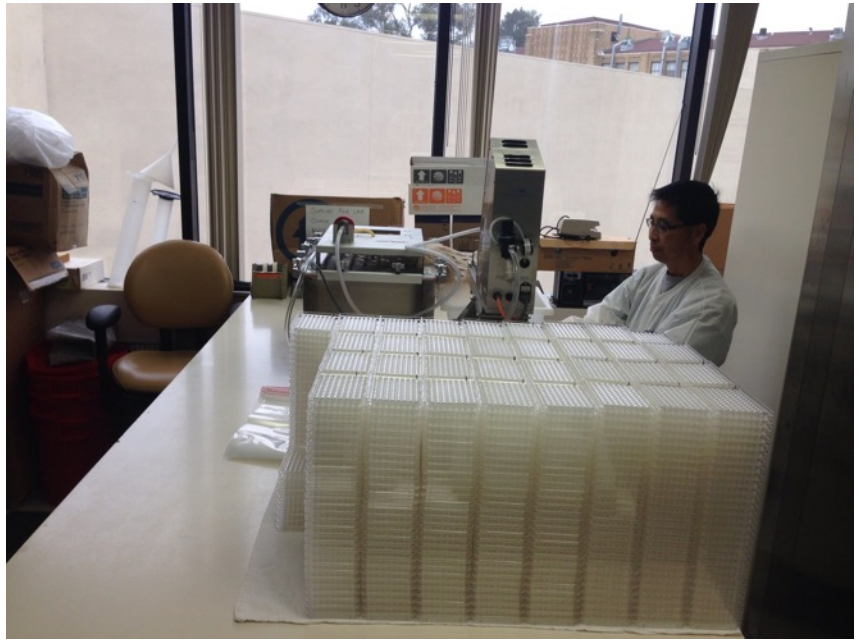
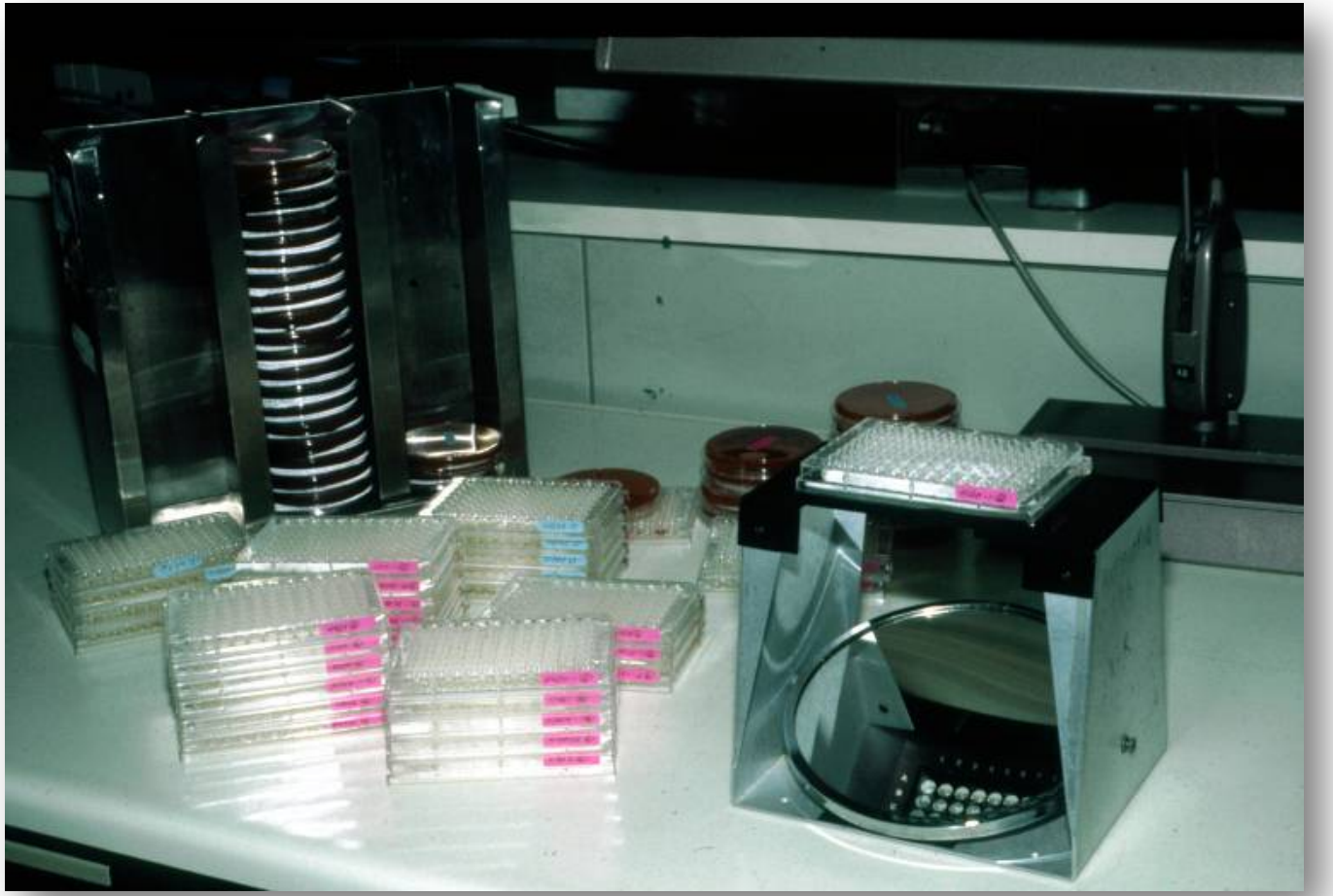
UCLA Experience

- Perform MIC testing, by the CLSI / ISO reference broth microdilution method for all patients (excluding Enterobacteriaceae from urine)



UCLA Experience Cont.

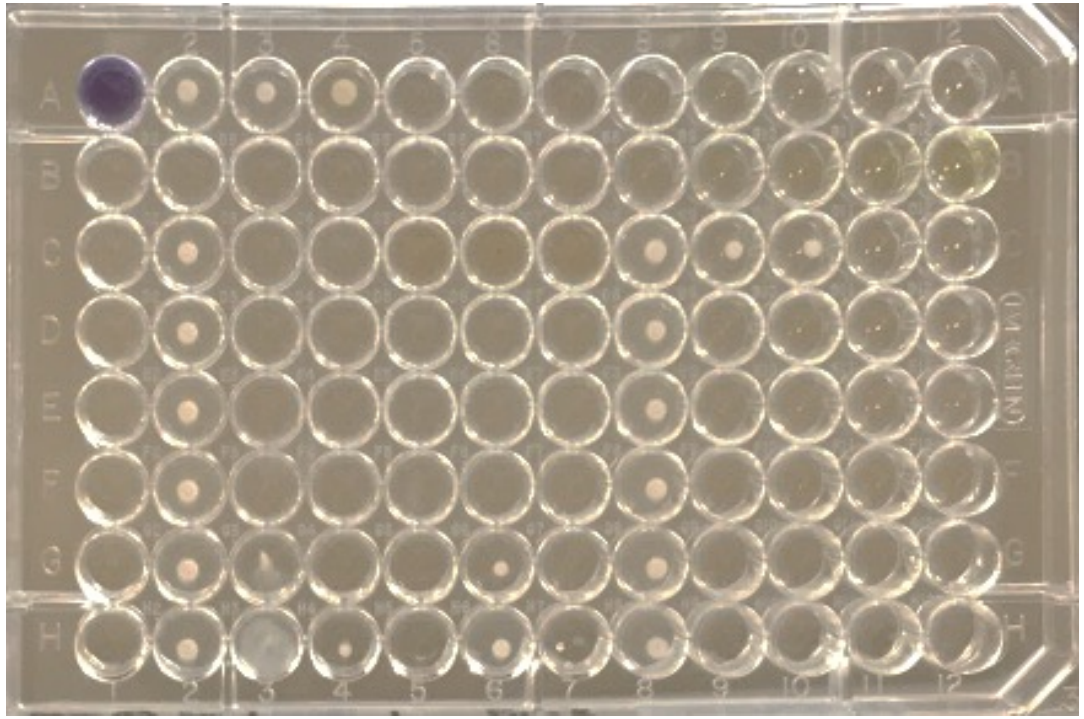


Routine Panels Manufactured at UCLA:

- Gram positive
- Gram negative
- Broad spectrum
- Fastidious (lysed horse blood)
- Yeast
- Nocardia / Rapidly growing AFB

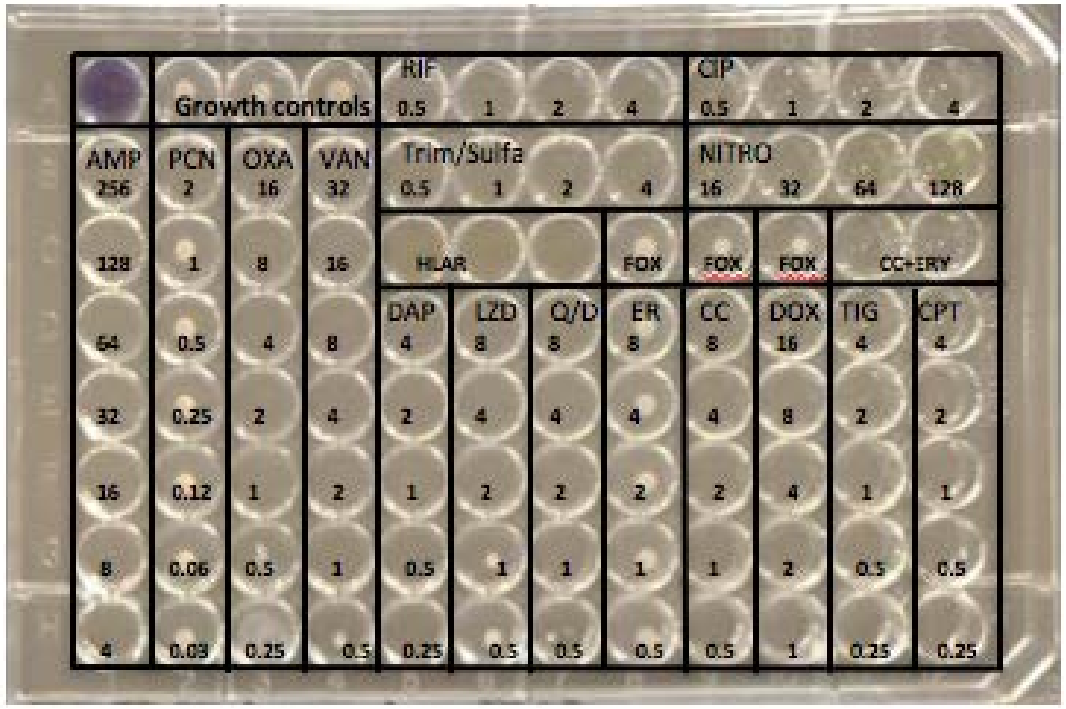
- Custom MIC for off-panel drugs

Reference BMD Panel



- CLSI method (ISO) = reference standard
- Well standardized
  - Media, inocula, testing conditions, etc
- … but still some variability

Reference BMD Panel



- CLSI method (ISO) = reference standard
- Well standardized
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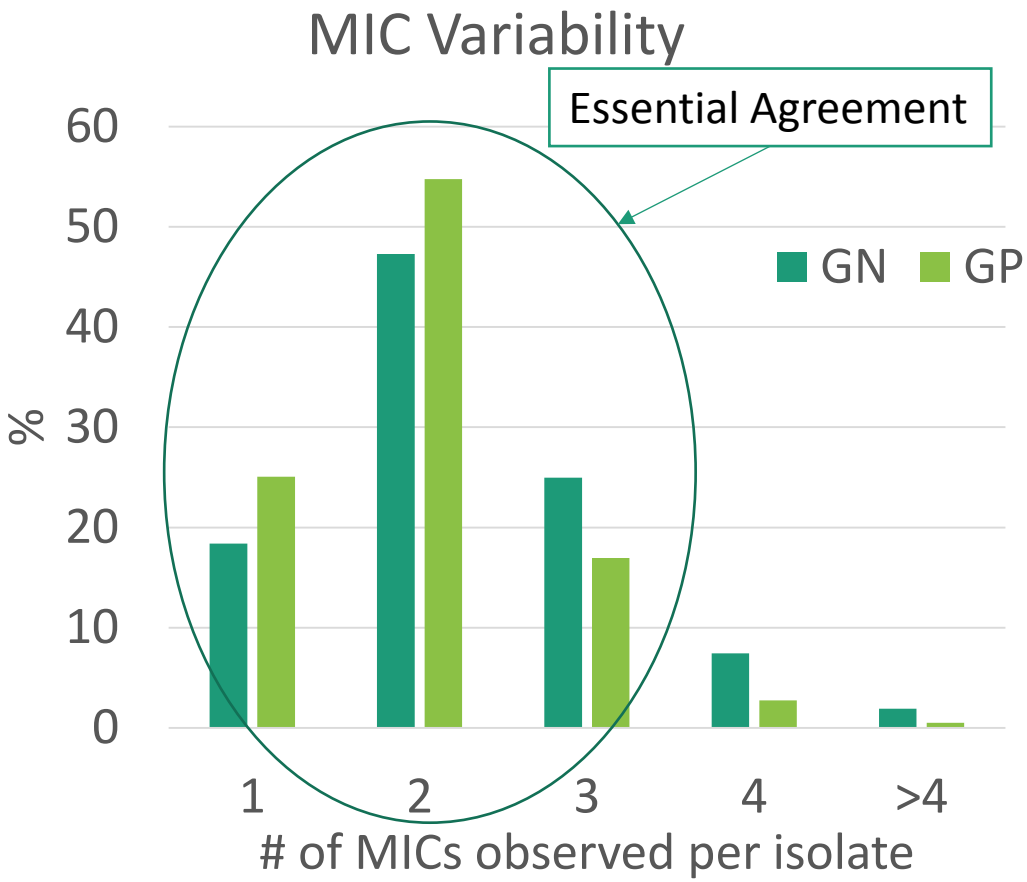
- … but still some variability

Variability of the BMD reference method

- 1 lab
- 9 replicates for each drug/bug combination
  - 3 people, 3 days
  - Same lot of panels
  - 91 GNR, 79 GPC all clinical isolates
  - 1927 bug/drug combos

Simulation from these pooled data → Sample size of 50, expect EA 88-100% of time

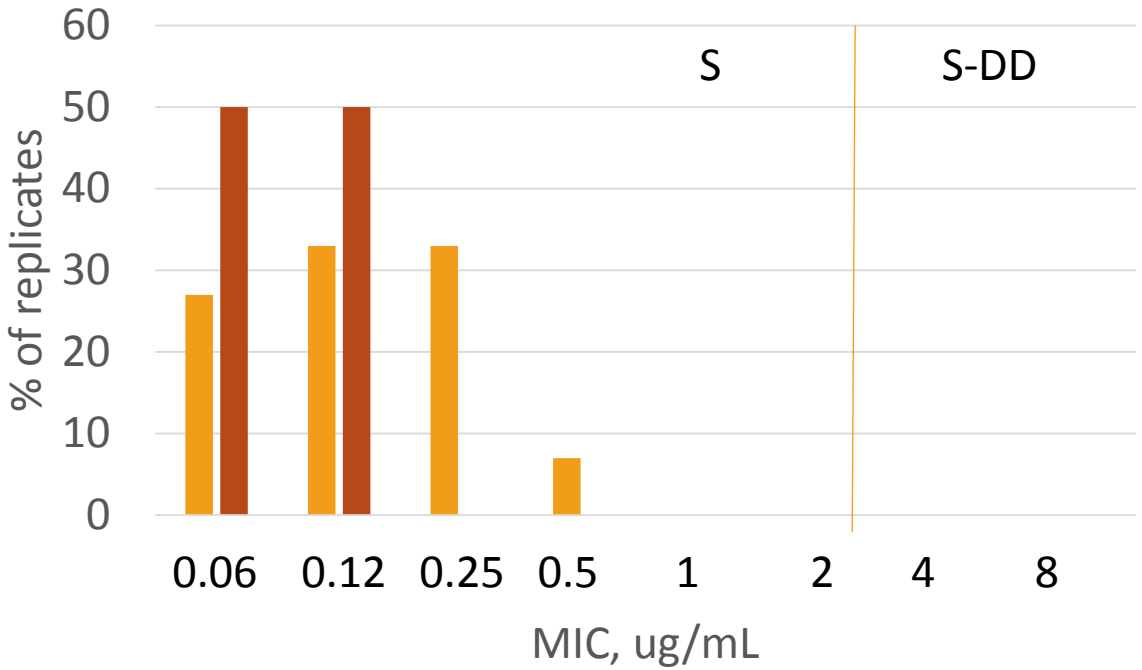


CLSI June 2016 Agenda Book, Data from bioMerieux

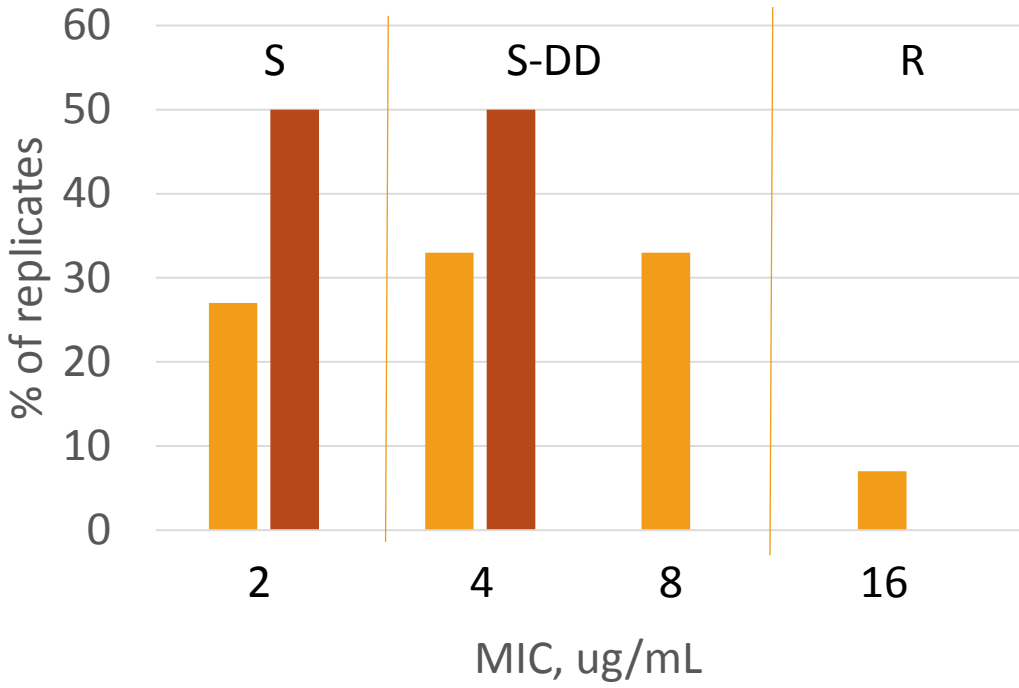
What does it mean?

- Unrealistic to think most organisms have a single or “real” MIC
- Some strains behave well, others less so
- How close to the breakpoint is the MIC?

Cefepime MIC for 2 isolates with “WT” MICs ~0.12 ug/mL



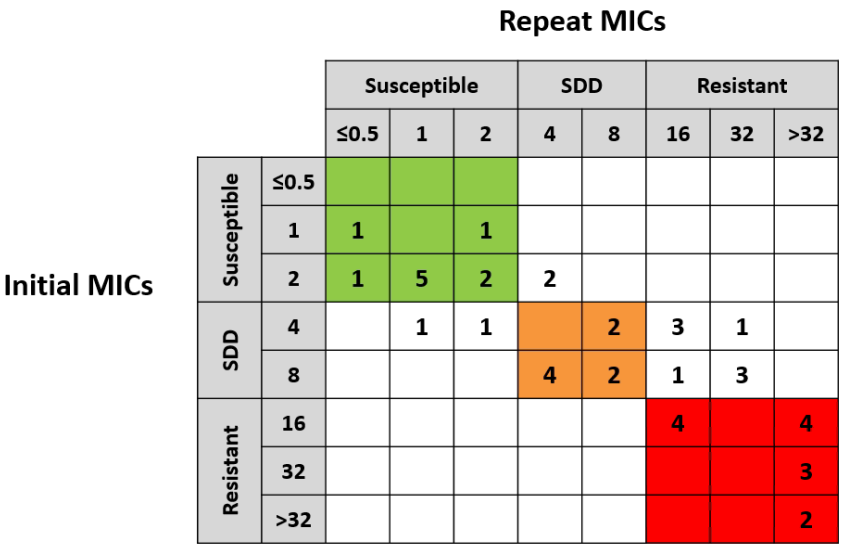
Cefepime MIC for 2 isolates with MICs ~4 ug/mL



Real-world data

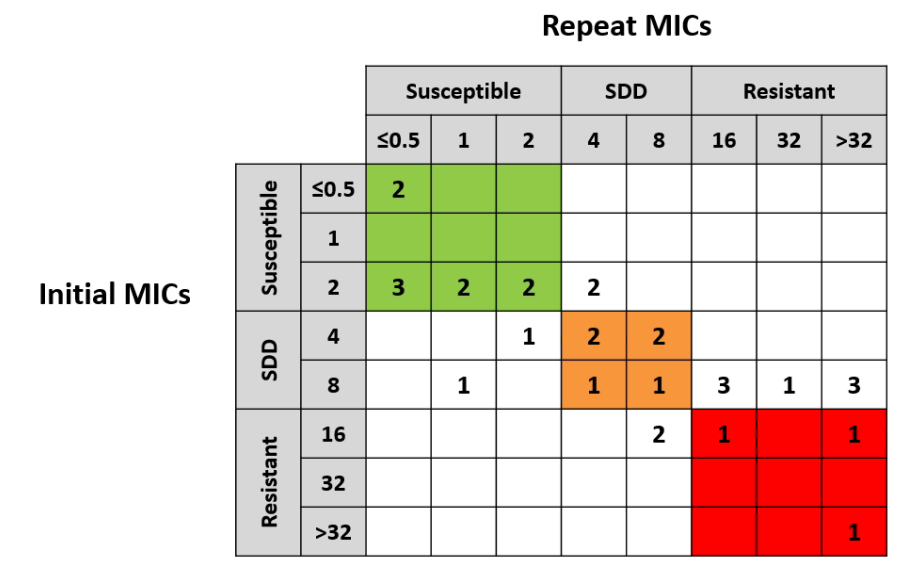
UCLA, clinical patient isolates. Tested by reference BMD on day 1 (reported to chart). Repeated BMD on day 2

E. coli spp; N=43



8/18 S-DD results repeated as S-DD (44%)
8/18 repeated as R (44%)
2/18 repeated as S (12%)

Klebsiella spp; N=31



6/15 S-DD results repeated as S-DD (40%)
7/15 repeated as R (47%)
2/15 repeated as S (13%)

Miller. Presented at ICAAC, 2014

Comparing commercial tests to BMD

J Clin Microbiol. 2014 Feb;52(2):392-7. doi: 10.1128/JCM.02432-13. Epub 2013 Nov 13.

Performance of Vitek 2 for antimicrobial susceptibility testing of Staphylococcus spp. and Enterococcus spp.

Bobenchik AM[1], Hindler JA, Giltner CL, Saeki S, Humphries RM.

- 2,950 drug-bug combos evaluated by BMD vs. Vitek2
  - 88 errors → 45 resolved on repeat testing
    → 38/45 errors due to initial BMD result (84%)

J Clin Microbiol. 2015 Mar;53(3):816-23. doi: 10.1128/JCM.02697-14. Epub 2014 Dec 24.

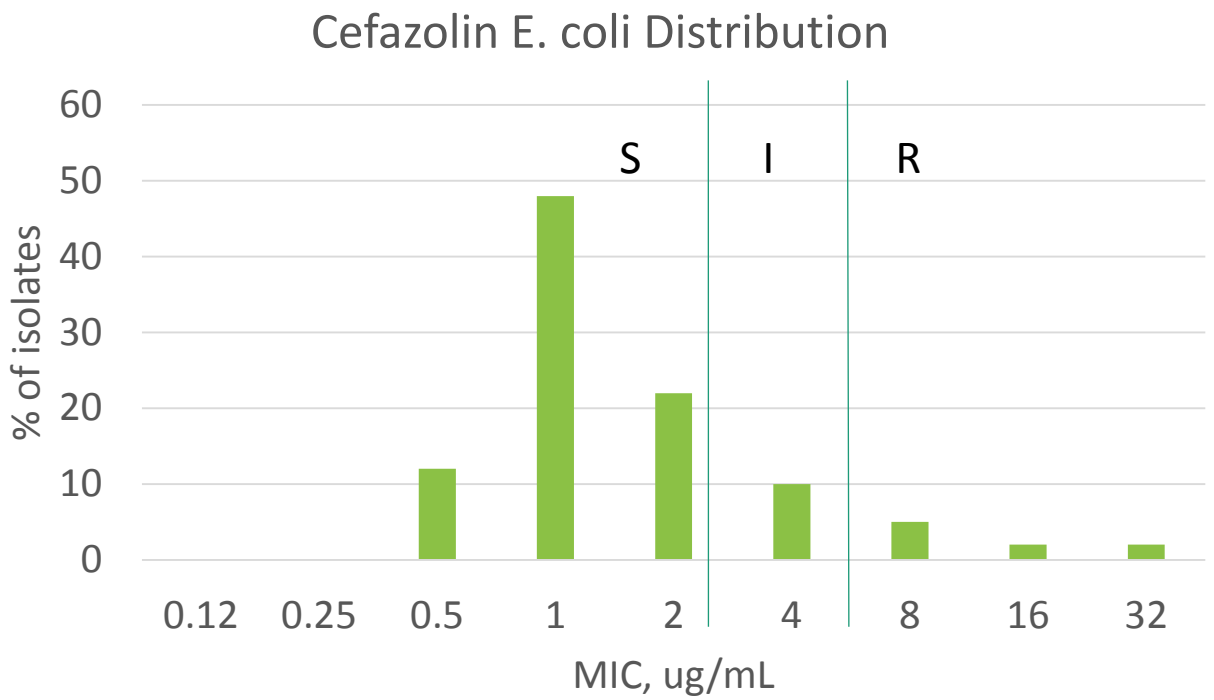
Performance of Vitek 2 for antimicrobial susceptibility testing of Enterobacteriaceae with Vitek 2 (2009 FDA) and 2014 CLSI breakpoints.

Bobenchik AM[1], Deak E[1], Hindler JA[2], Charlton CL[1], Humphries RM[3].

- 6,244 drug-bug combos evaluated by BMD vs. Vitek2
  - 37 errors → 21 resolved on repeat testing
    → 6/21 errors due to initial BMD result (29%)

How about if we push the breakpoint into the wild-type distribution?

- Example: Cefazolin & Enterobacteriaceae



Problem: every isolate tested is likely to be within 2 dilutions of BP!

- No longer routinely test at UCLA
- No commercial manufacturer has updated this breakpoint on their systems

How about if there is no “buffer” zone? (intermediate)

- **Intermediate (I) – a category defined by a breakpoint that includes isolates with MICs or zone diameters within the intermediate range, that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates; NOTE: The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated or when a higher than normal dosage of a drug can be used. This category also includes a buffer zone, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins.**

- Intermediate isn’t just for alternative dosing

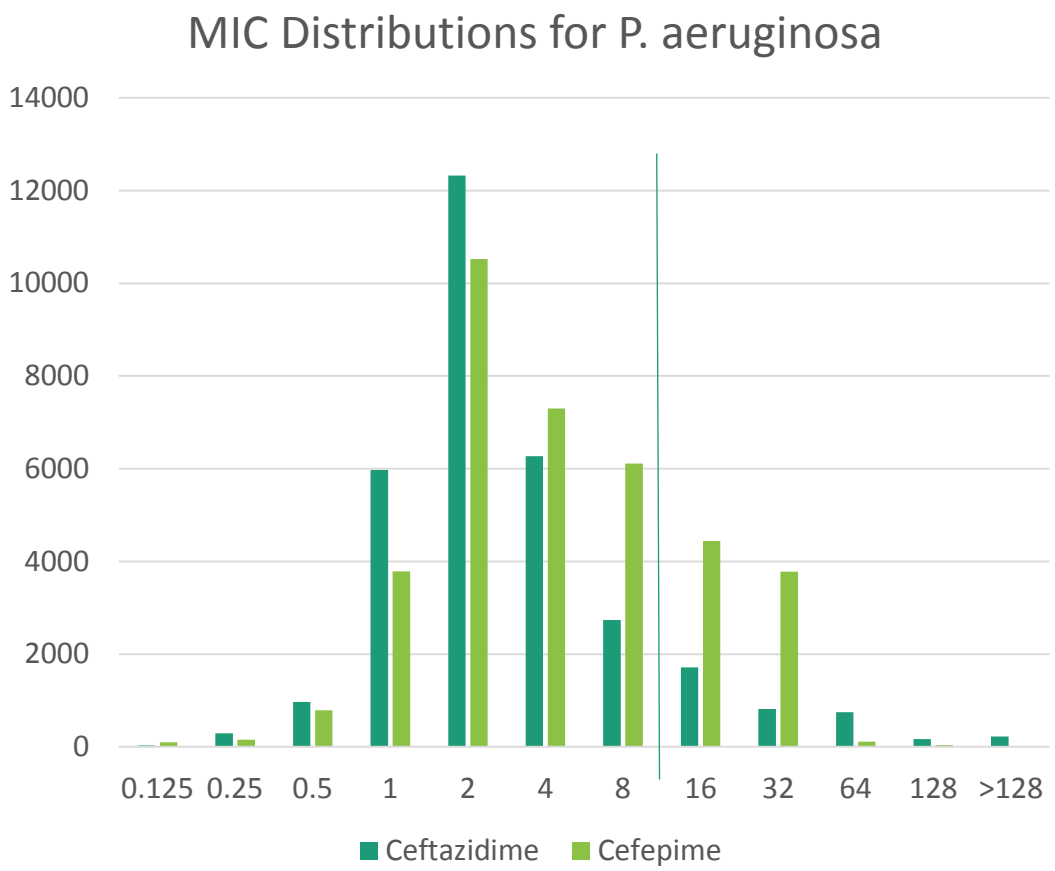
Examples of CLSI breakpoints with no “I” (GNR):

- folate pathway inhibitors
- polymyxins (*A. baumannii*, *P. aeruginosa*)

Example: cefepime and ceftazidime vs. *P. aeruginosa*

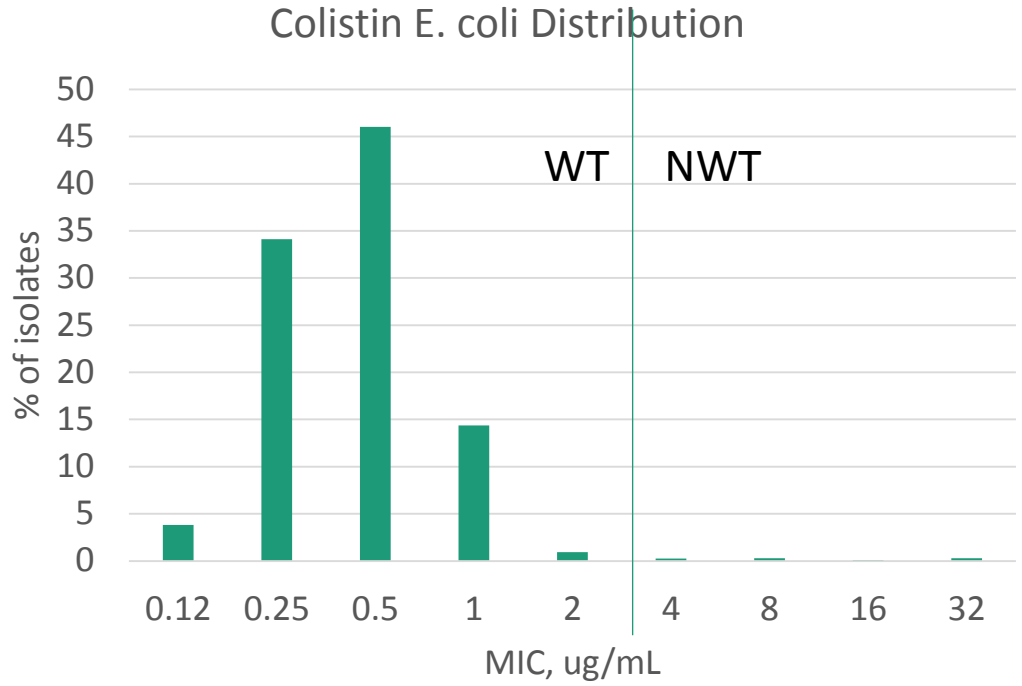
Breakpoints for cefepime & ceftazidime

	S	I	R
FDA	<=8	-	>=16
CLSI	<=8	16	>=32



How about if we do both? Push to WT and have no buffer?

- Example: Colistin & Enterobacteriaceae



Experiment performed at UCLA.
One panel, 6 replicate wells of colistin, 3 brands CA-MHB

MIC (ug/mL)	E. aerogenes #1	E. coli # 1 (mcr-1)	E. coli #2 (WT)
0.5	2		3
1.0			3
2.0		2	
4.0	2	4	
>=8.0	2		
% “WT”	33%	33%	100%

Putting it in context…

- “Gold standard” BMD isn’t a “bad” method – usually reproducible within 1 dilution
- Can be challenging if the ‘wild type’ MIC of a given species is near breakpoint (example: cefazolin)
- Can be challenging if there is no “buffer” zone (intermediate)
- May need different acceptance criteria for MICs near BP, or if there is no intermediate BP
- May need to allow discrepancy resolution in submission process
- May need to further evaluate BMD method variability for new drugs and use data in context of FDA submissions

Back to colistin for a minute….

- Not a single FDA-cleared AST test for colistin is available
- Why? No FDA breakpoint
- Biggest use in US: CRE
  - No CLSI breakpoint either!
  - CLSI established an ECV (epidemiological cut-off value) for colistin due to lack of PK/PD and clinical data
  - Could we have tests cleared with ECVs only?

Clinical Breakpoint	ECV
- Based on PK/PD, clinical outcomes and MIC distributions	- Based on MIC distributions
- Used to predict likelihood of clinical success, by MIC	- Used to predict emergence of resistance, by MIC

The problem with breakpoints (2017)

- “old” tests that were cleared before FDA started enforcing FDA breakpoint rule (i.e., 2007) can report what they like
  - Example: some systems can test *E. faecium* vs. daptomycin, others cannot

- Why is this a problem?

1) new technologies at a disadvantage with FDA

2) issues cannot be fixed, as system will be able to report fewer drug/bug combos post-resubmission

3) breakpoints are challenging to update as system may loose some capability

Accelerate Example…

Antimicrobials tested on Pheno system for GNR

	SAM	TZP	FEP	CAZ	CRO	ERT	MEM	AK	GM	NN	CIP	ATM	COL
Pheno Reportable		Y						Y					
CLSI BP (old systems)	Y	Y	Y	Y	Y	n/a	Y	Y	Y	Y	Y	n/a	Y/N

Y, can report

No FDA BP for A.baumannii

Clinical Indications vs. Breakpoints…

- FDA will only approve a test for a drug/bug with clinical indication

- EXAMPLE: Ceftazidime-avibactam

Clinical Indications	**cUTI: *E. coli, K. pneumoniae, C. koseri, E. aerogenes, E. cloacae, C. freundii, Proteus, P. aeruginosa* IAI: *E. coli, K. pneumoniae, P. mirabilis, E. cloacae, K. oxytoca, C. freundii, P. aerguinosa***
Breakpoints	Enterobacteriaceae P. aeruginosa

No test could include *Serratia* or *Providencia* in FDA submission

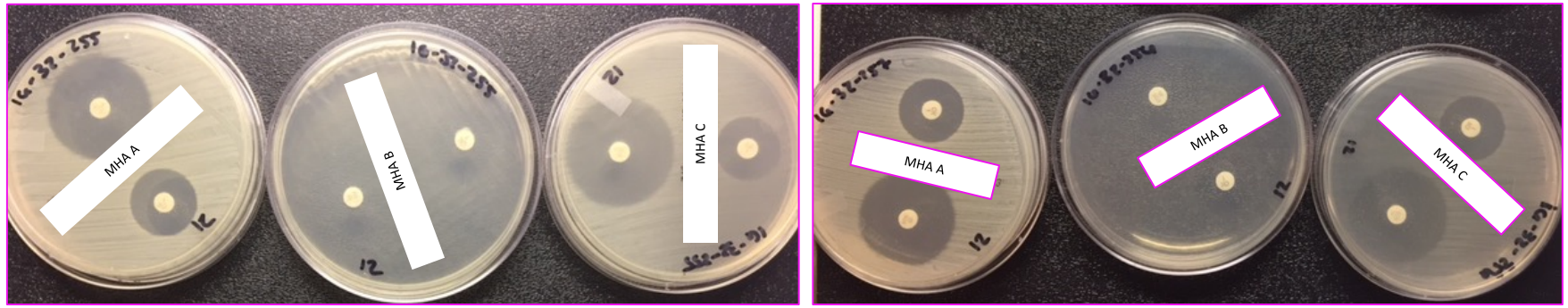
Changes to breakpoints:
21st Century Cures Act – passed in 2016

Effective November 2017:
- remove breakpoints from drug label
- FDA to recognize breakpoints set by standard setting organizations (e.g., CLSI)
- Device manufacturers can get clearance for devices with these BPs

- Hope: this can help address this dilemma (will it?)

One more challenge: the other CLSI reference method

- Disk diffusion also CLSI reference method
- Many more laboratories use this in clinical practice vs. BMD
- Disks are FDA-cleared (performance demonstrated)
  - But: only require use of 1 brand MHA in submission
  - Breakpoints also set using 1 brand MHA/ 1 lab
- Manufacturing of disks regulated → acceptable limits set by FDA
- Media to follow ISO standard… but differences?



Miller. Data Submitted to CLSI Agenda Book, June 2017