
Welcome!
2025 WCLN Conference:
"Let's Talk: Antimicrobial Resistance, Stewardship, and Susceptibility Testing"
Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN–MADISON

Thank you for being here!

- Help yourself to refreshments
- Introduce yourself to your neighbor

Appreciation

- **LabTAG**
  - For all you support and guidance in planning this conference
- **Speakers**
  - For sharing your knowledge, talents, and your valuable time
- **WCLN**
  - For your participation and enthusiasm and all the hard work that you do
- **Wilderness Glacier Canyon Lodge Staff**
  - For hosting us and providing us this lovely venue to communicate our science
- **Jim Hermanson**
  - For assisting with the technological development and planning

Today's Speakers

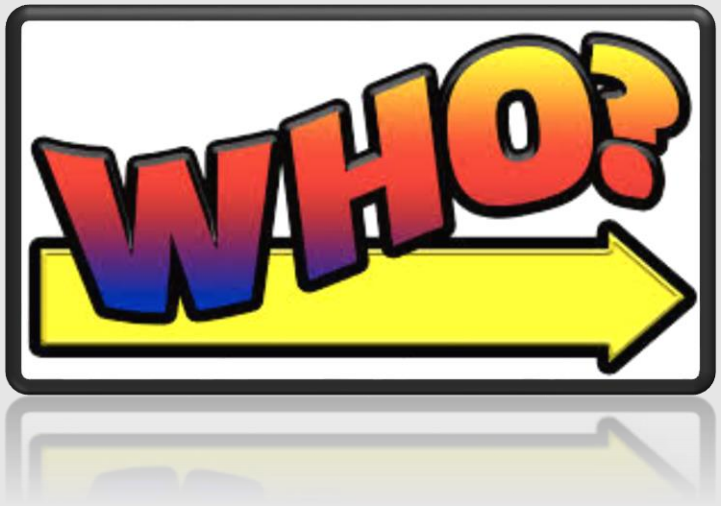
- **Heather Alvarez, MS, CLS(ASCP),** Laboratory Manager, Prairie Ridge Health, Columbus
- **Alexander Lepak, MD, FIDSA,** Associate Professor of Medicine, Medical Director, Chair of the Antimicrobial Use Committee, UW Health, University of Wisconsin School of Medicine and Public Health
- **Rachael Liesman, PhD, D(ABMM),** Director Clinical Microbiology & Molecular Diagnostics, Wisconsin Diagnostic Laboratory, Milwaukee
- **Erik Munson, PhD, D(ABMM),** Assistant Professor, College of Health Sciences, Marquette University, Milwaukee
- **Alana Sterkel, PhD, D(ABMM), SM(ASCP)^{CM},** Associate Director, Communicable Disease Division Wisconsin State Laboratory of Hygiene, Madison
- **Virginia Pierce, MD, FIDSA,** Pediatric Infectious Disease Physician, Clinical Associate Professor, University of Michigan, Ann Arbor
- **Taylor Wahlig, PhD, D(ABMM),** Technical Director of Microbiology and Molecular Pathology, Marshfield Clinic Health System, Marshfield
- **Macy Wood, PhD, D(ABMM),** Assistant Professor, Associate Director of Clinical Microbiology, Froedtert Health/Wisconsin Diagnostic Lab, Milwaukee
- **Caitlin Cahak, MLS (ASCP)^{CM},** Microbiology Technical/Administrative Supervisor, Froedtert Health/Wisconsin Diagnostic Laboratory, Milwaukee
- **Megan Selle, MLS, M(ASCP),** Microbiology Supervisor, ThedaCare Regional Medical Center, Neenah
- **Will Laudon, BA, MB(ASCP),** Microbiology Technical Specialist, Wisconsin Diagnostic Laboratory

We Hope You Enjoy the Day!

- Participate in discussions
- Help us learn by sharing your experience and knowledge
- Sit by, or have lunch with someone you don't know and make a new contact.

Overview of WCLN Conferences

Who Plans the WCLN Conferences?



- WCLN is a Collaborative Network - WSLH relies on LabTAG guidance.
- Needs Assessment - LabTAG focuses on needs of the clinical laboratories
  - Based on their own laboratory experiences
  - Review comments and suggestions on program evaluations
- Diversity - LabTAG works hard to ensure all labs, no matter their size, have a voice and feel included in the WCLN
- Goal - Elevate all WCLN laboratories.
  - No lab gets left behind or is alone facing technology changes, updates, or challenges

What Topics Do We Focus On?

- Due to frequent changes in antimicrobial susceptibility testing (AST), we spend about every other year discussing updates to AST at our WCLN Technical Conference
- Realized microbiology technology was changing and held our first spring technical conference in 2009 on Molecular Diagnostics
  - 11 laboratories presented information on molecular testing they were performing.
- In 2011 we continued the discussion highlighting available molecular platforms as well as other developing technologies such as Maldi-TOF
  - Discussed the pros and cons
  - Validation, verification, QC and PT
  - The sales pitch to administration
- In 2013 we brought quality into the discussion by asking how do we provide quality laboratory services.
- In 2014 started talking about the future of automation and how new technology will impact patient care
- In 2016 we first discussed syndromic multiplex panels and waived PCR testing

2025 Peter A. Shult Award Winner

A Susceptibility Testing Catch-22: Applying Current Breakpoints under the Shadow of the FDA's New LDT Rule

Wisconsin Clinical Laboratory Network (WCLN) Spring Technical Conference

Wisconsin Dells, WI • April 1, 2025

Virginia M. Pierce, MD, FIDSA
Medical Director, Clinical Microbiology Laboratory
Clinical Associate Professor of Pathology and Pediatrics (Pediatric Infectious Diseases)
vpierce@med.umich.edu

Ongoing legal challenges to the FDA LDT Rule

The American Clinical Laboratory Association (ACLA) and the Association for Molecular Pathology (AMP) filed lawsuits challenging the FDA's authority to regulate LDTs

Oral arguments were heard 2/19/25 in a US District Court in Plano, Texas – since then, we have been awaiting the judge's decision

Some legal experts had expected the Trump administration to walk back the FDA LDT Rule and were surprised that the DOJ counsel representing HHS defended the rule (as it had during the Biden administration)

- Is this truly the administration's position? Or have they been so active in other areas that they did not have time to reformulate their policy and prepare a new oral argument?

https://www.aruplab.com/news/02-20-2025/acla-amp-ask-federal-court-strike-down-fda-rule
https://www.raps.org/news-and-articles/news-articles/2025/2/trump-administration-s-defense-of-ldt-rule-catches

On 3/31/25 (a.k.a. yesterday!), we got an answer

UNITED STATES DISTRICT COURT
EASTERN DISTRICT OF TEXAS
SHERMAN DIVISION

AMERICAN CLINICAL LABORATORY ASSOCIATION, ET AL. v. U.S. FOOD AND DRUG ADMINISTRATION, ET AL.	§ § § § § § § §	CIVIL NO. 4:24-CV-479-SDJ
ASSOCIATION FOR MOLECULAR PATHOLOGY, ET AL. v. U.S. FOOD AND DRUG ADMINISTRATION, ET AL.	§ § § § § § § §	CIVIL NO. 4:24-CV-824-SDJ

MEMORANDUM OPINION AND ORDER

Ordered that the Final Rule on LDTs be vacated, noting that the FDA lacked the authority to regulate LDTs

Excerpt from judge's 51-page ruling:

FDA's asserted jurisdiction over laboratory-developed test services as "devices" under the FDCA defies bedrock principles of statutory interpretation, common sense, and longstanding industry practice. The FDCA—a statute targeted at mass-produced, mass-marketed, and mass-distributed drugs and devices moving in interstate commerce—is a poor fit for the distinct regulatory issues raised by laboratories that provide vital diagnostic tools for doctors. Blinking this reality, FDA's final rule creates a "square peg into round hole" problem that is not just about a tortured reading of an unambiguous statute, or about FDA attempting to fill a regulatory gap or administer a statute in the face of congressional silence. The more fundamental problem is that Congress has already considered the distinct issues raised by laboratory-developed test services in CLIA, and chose to address those issues by vesting regulatory authority in CMS, not in FDA. Through the final rule, it appears that FDA is attempting to circumvent that legislative decision. It has no authority to do so.

Not yet known whether this decision will be appealed and/or if Congress will pass legislation in this space

New agenda

1. What is a breakpoint?
2. How are breakpoints set?
3. Why do breakpoints change?
4. Why should labs use current breakpoints?
5. How would the FDA's new LDT rule create a Catch-22 for labs?
6. How do we get out of this mess?!?

Question #1:
What is a breakpoint?

Escherichia coli

Antimicrobial	**MIC, μg/mL**
Ampicillin	≥ 32
Ampicillin-sulbactam	≥ 32/16
Piperacillin-tazobactam	16/4
Cefazolin	32
Ceftriaxone	16
Cefepime	4
Aztreonam	8
Ertapenem	0.5
Meropenem	≤ 1
Gentamicin	2
Amikacin	4
Ciprofloxacin	1
Tetracycline	8
Trimethoprim-sulfamethoxazole	≥ 4/76

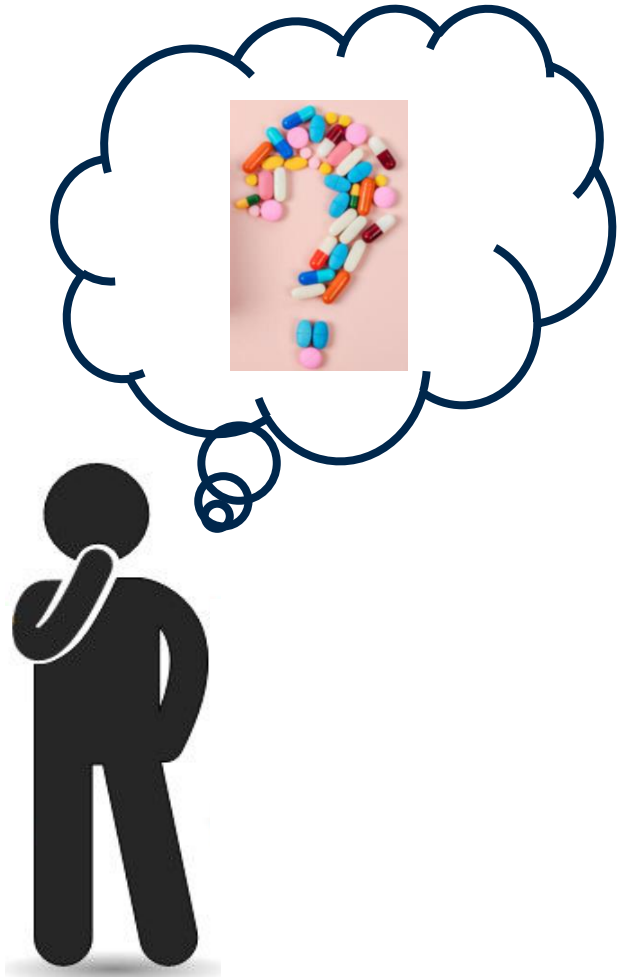
Escherichia coli

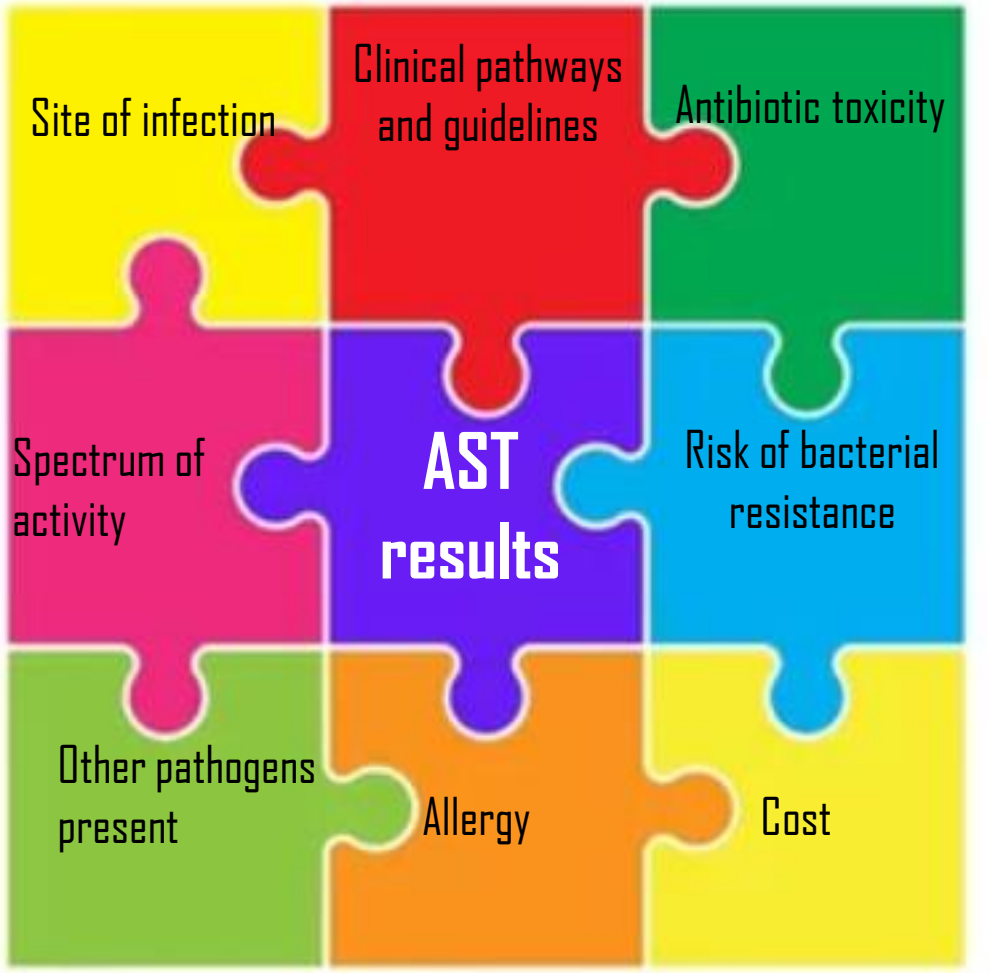
Antimicrobial	MIC, µg/mL	Interpretation
Ampicillin	≥ 32	Resistant
Ampicillin-sulbactam	≥ 32/16	Resistant
Piperacillin-tazobactam	16/4	Susceptible-Dose Dependent
Cefazolin	32	Resistant
Ceftriaxone	16	Resistant
Cefepime	4	Susceptible-Dose Dependent
Aztreonam	8	Intermediate
Ertapenem	0.5	Susceptible
Meropenem	≤ 1	Susceptible
Gentamicin	2	Susceptible
Amikacin	4	Susceptible
Ciprofloxacin	1	Resistant
Tetracycline	8	Intermediate
Trimethoprim-sulfamethoxazole	≥ 4/76	Resistant

Breakpoints are predictions

- Minimal inhibitory concentration (or zone diameter) interpretive cutoffs used to **predict the likelihood of a successful clinical outcome** if a particular antimicrobial is prescribed

Antimicrobial susceptibility testing (AST) results are highly influential in prescribing decisions





Who sets breakpoints?

CLSI

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

USCAST

FDA U.S. FOOD & DRUG ADMINISTRATION

There are some differences in the specifics of how each organization approaches breakpoint setting

BUT

overall, there really is more that's the same than there is that's different

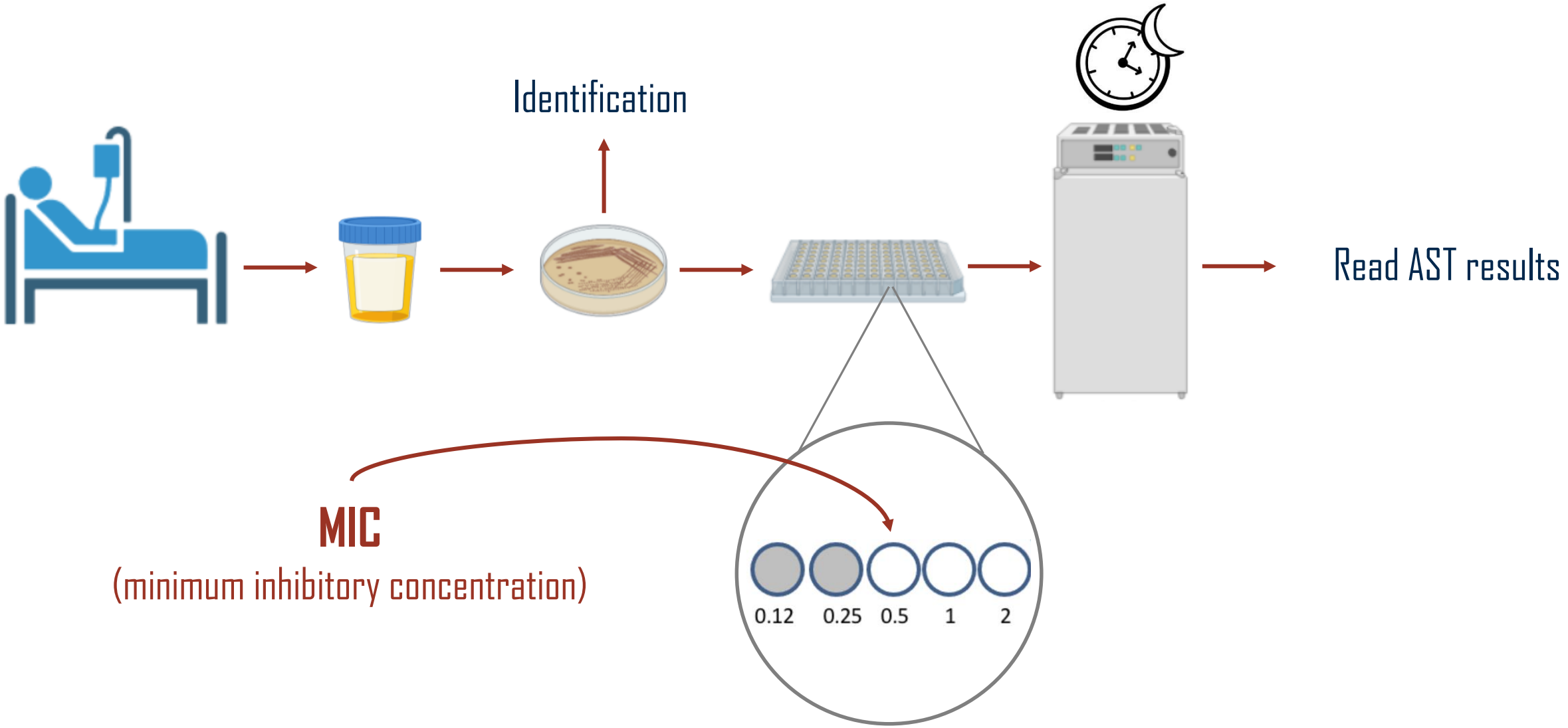
Question #2:
How are breakpoints set?

Types of data weighed when setting breakpoints

1. Microbiological data
2. Pharmacokinetic-pharmacodynamic (PK-PD) data
3. Clinical data

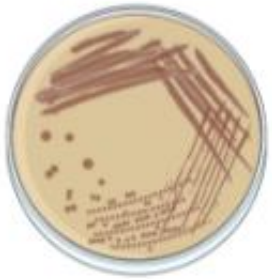
Category #1: Microbiological Data

Broth microdilution (gold standard for AST)



Icons from biorender.com

Goal: determine whether an isolate belongs to the "wild-type" or "non-wild-type" population



?

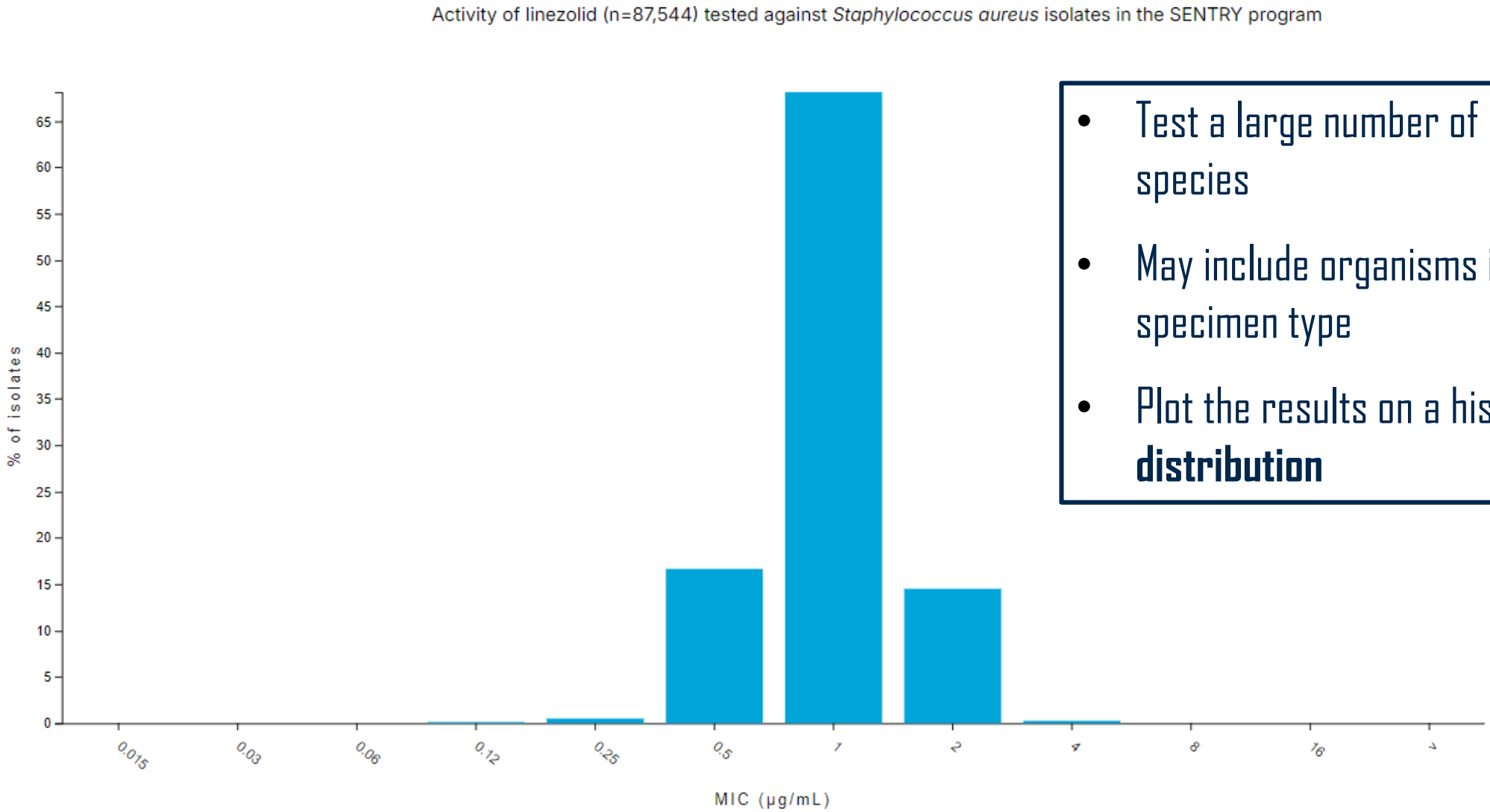
?

Wild-type:
isolates without any acquired resistance to the antimicrobial in question

Non-wild-type:
isolates that have acquired resistance to the antimicrobial

https://imageresizer.com/meme-generator/edit/fancy-winnie-the-pooh-meme

What if we generate a **lot** of MICs?



- Test a large number of isolates of a single species
- May include organisms isolated from any specimen type
- Plot the results on a histogram → **MIC distribution**

https://sentry-mvp.jmilabs.com/

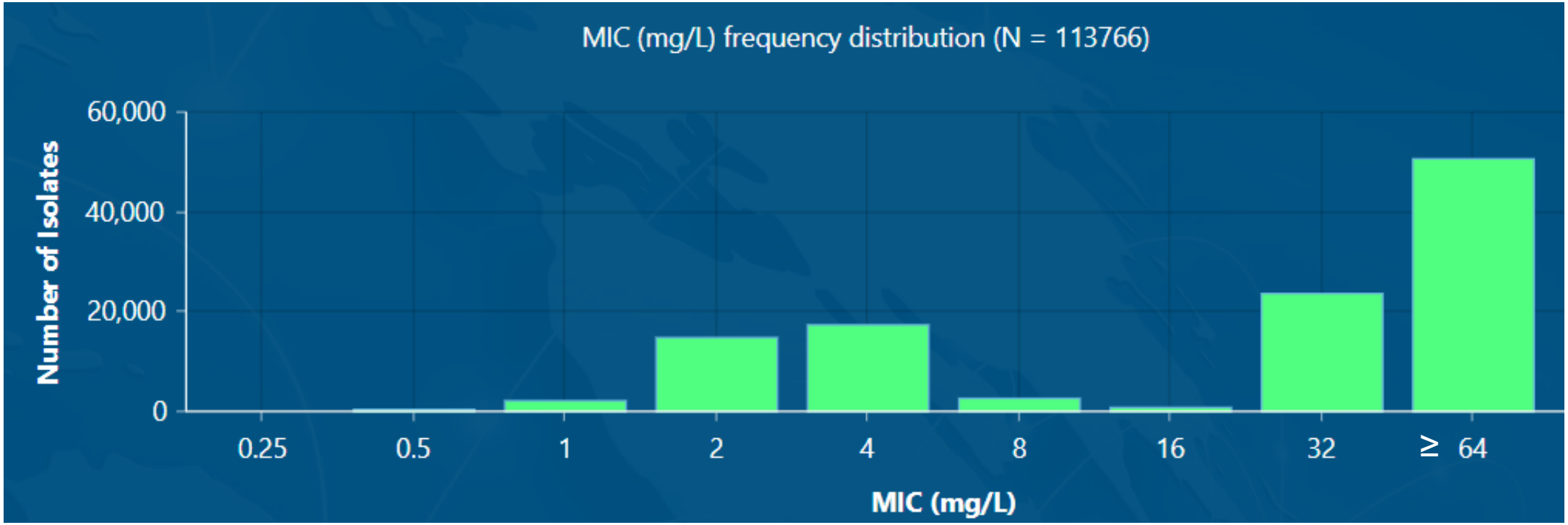
Why aren't the MICs for wild-type organisms all exactly the same?

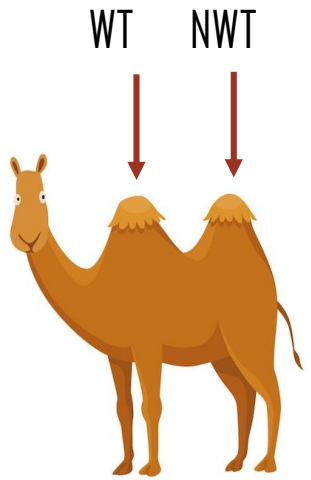
- Even under the best controlled testing conditions, the combination of an isolate's inherent biological variability taken together with the technical variability of the assay leads to a **range** of MIC values with replicate testing

- The MIC is often within a 3-dilution (± 1 doubling dilution) range, but sometimes this can be even wider

What does this MIC distribution show?

Escherichia coli and ampicillin





https://atlas-surveillance.com/

Epidemiological cutoff value (ECV)

- ECV = the MIC that separates microbial populations into those without and those with acquired resistance based on their phenotypes (wild-type or non-wild-type)
  - What value defines the upper end of the wild-type MIC distribution, such that the MICs for 97.5% of WT isolates fall at or below that value?
- There are specific criteria for how to formally set an ECV
  - General concepts: single species, reliable AST method, lots of isolates, multiple participating laboratories, data are not truncated within the wild-type distribution
  - Iterative statistical method used to arrive at the cutoff

How does the ECV factor into the breakpoint?

- **ECV ≠ Breakpoint**
  - Only describes the MIC distribution
  - Does not account for the other two important categories of data (PK-PD and clinical)
  - Does not predict clinical response

How does the ECV factor into the breakpoint?

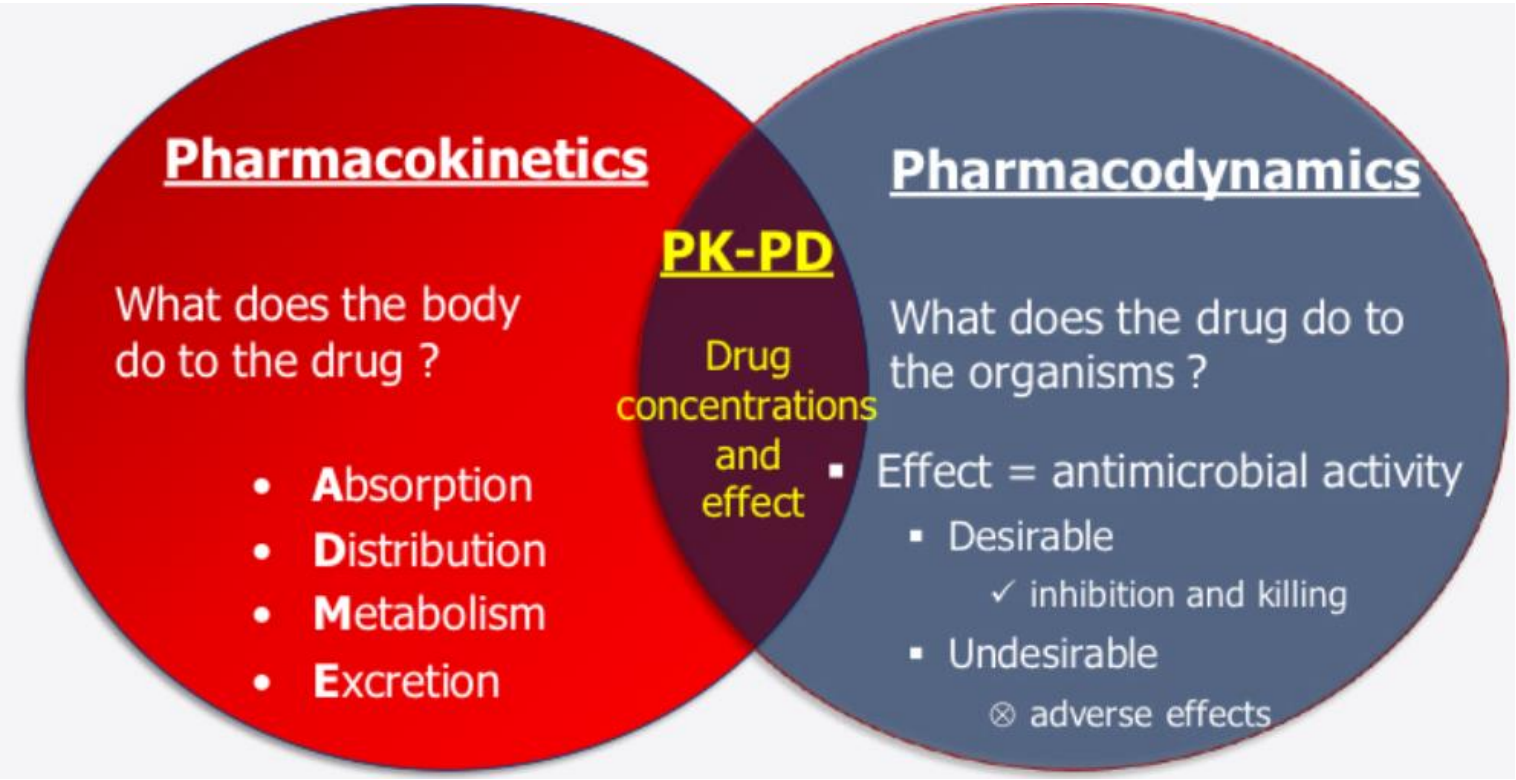
Important to avoid setting breakpoints within the wild-type MIC distribution (i.e., lower than the ECV)

- If a breakpoint splits the wild-type distribution, we are asking our susceptibility tests to differentiate between organisms that are part of the same population and are not actually meaningfully distinct from one another
  - The flip-flop between S, I, and R may be frequent and random
  - An individual AST result may not be reliable
  - AST device manufacturers will have difficulty making tests that perform well enough to get FDA clearance → most clinical labs will not be able to offer AST

Category #2: PK-PD Data

Interplay between PK and PD of antimicrobials

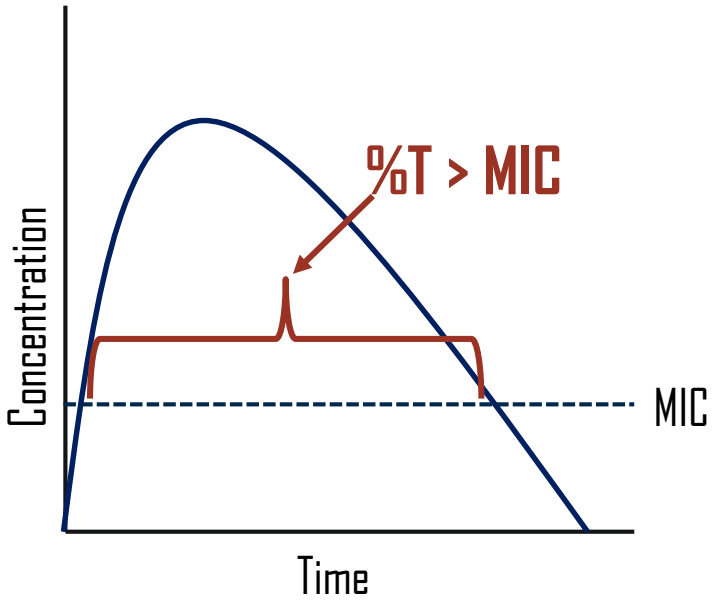
What are the achievable (free) drug levels in blood and other body fluids?

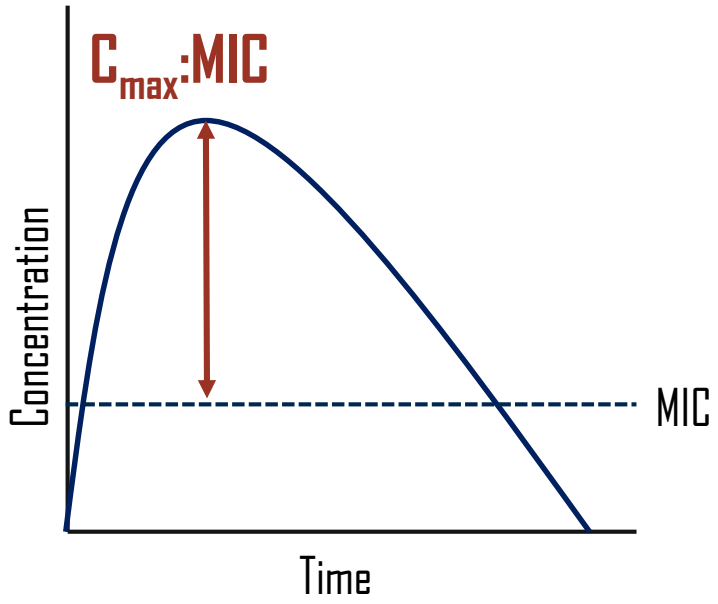


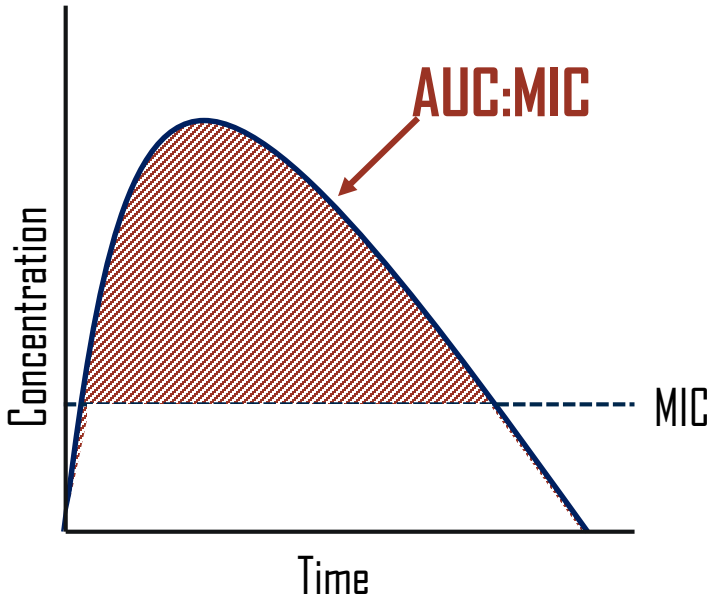
What is the relationship between (free) drug concentration over time (exposure) and antimicrobial effect?

https://clsi.org/media/1990/ast_news_update_jan18-pkpd.pdf

What PK-PD index is most closely linked to antimicrobial effect?







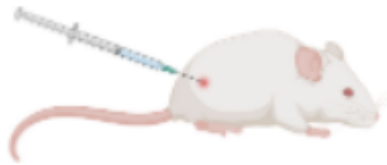
PK-PD target:

The **magnitude** of that PK-PD index at which a desired level of response is achieved

Neutropenic mouse thigh infection model

Cyclophosphamide

Bacteria



use strains with MICs and resistance mechanisms we expect to encounter clinically, considering the intended use of the drug

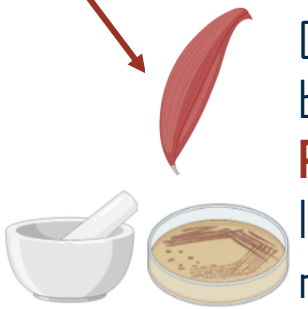
Antibiotic

human-simulated antibiotic regimens

Pharmacokinetics:

$t_{1/2}$
C_{max}
AUC
Clearance…

Mortality?



Pharmacodynamics:
Colony-forming units (CFU) of bacteria →
PK-PD target (for stasis? for a 1-log reduction? for a 2-log reduction?)

Icons from biorender.com

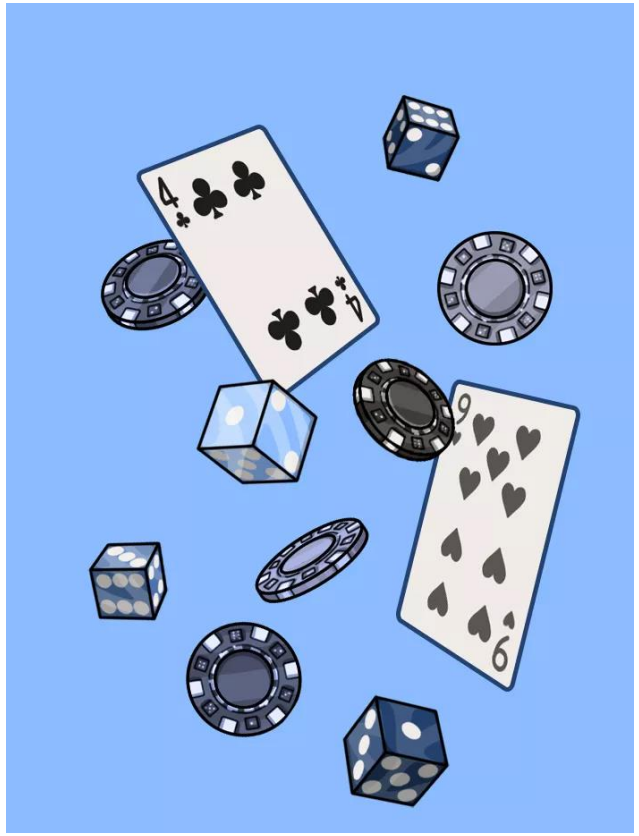
What about PK data in humans?

- **Range of exposures** to the antimicrobial agent that are achieved following administration of the selected dosage regimens in **target patient populations**

Variable	*n*	Mean	SD	Median	Min	Max
Total C_{max} (mg/liter)	55	2.58	1.33	2.24	0.539	7.88
Unbound C_{max} (mg/liter)	55	0.749	0.364	0.629	0.238	2.21
T_{max} (h)	55	1.02	0.0848	1.00	0.750	1.48
Total AUC_{0-24} (mg · h/liter)	55	24.3	7.88	22.8	8.09	50.9
Total $AUC_{0-\infty}$ (mg · h/liter)	55	46.6	19.7	44.4	15.1	96.7
Unbound AUC_{0-24} (mg · h/liter)	55	7.18	2.46	7.12	2.74	13.3
Unbound $AUC_{0-\infty}$ (mg · h/liter)	55	14.1	6.68	13.7	3.65	29.2
CL (liters/h)	55	5.24	2.63	4.50	2.07	13.2
V_{ss} (liters)	55	146	57.0	140	54.7	465
$T_{1/2,\alpha}$ (h)	55	1.36	0.456	1.35	0.448	3.44
$T_{1/2,\beta}$ (h)	55	23.4	9.53	20.3	8.87	46.8
f_{ub}	55	0.309	0.120	0.280	0.159	0.957

Lodise TP et al. Pharmacokinetic and pharmacodynamic profiling of minocycline for injection following a single infusion in critically ill adults in a phase IV open-label multicenter study (ACUMIN). Antimicrob Agents Chemother 2021; 65:e01809.

Dealing with variability: Monte Carlo simulation

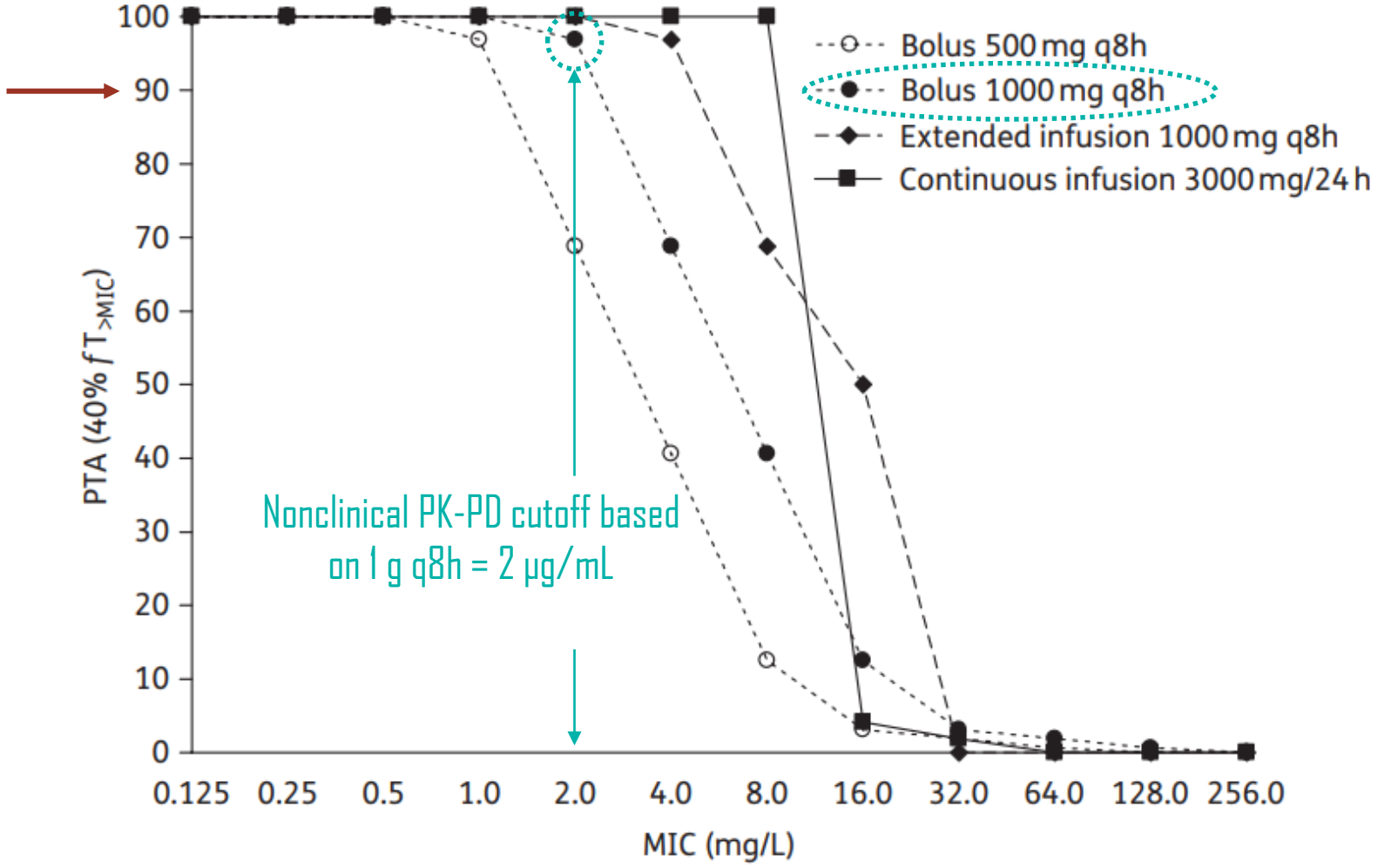


- A model that uses repeated random sampling to predict the probability of various outcomes when the input values are variable
- Estimate the **probability of attaining the PK-PD target for efficacy at different MICs**

https://www.investopedia.com/terms/m/montecarlosimulation.asp. Mouton JW. Setting clinical breakpoints from a PK/PD point of view: it is the dose that matters. In Fundamentals of Antimicrobial Pharmacokinetics and Pharmacodynamics, 2014. Roberts JA et al. J Antimicrob Chemother 2010; 66: 227.

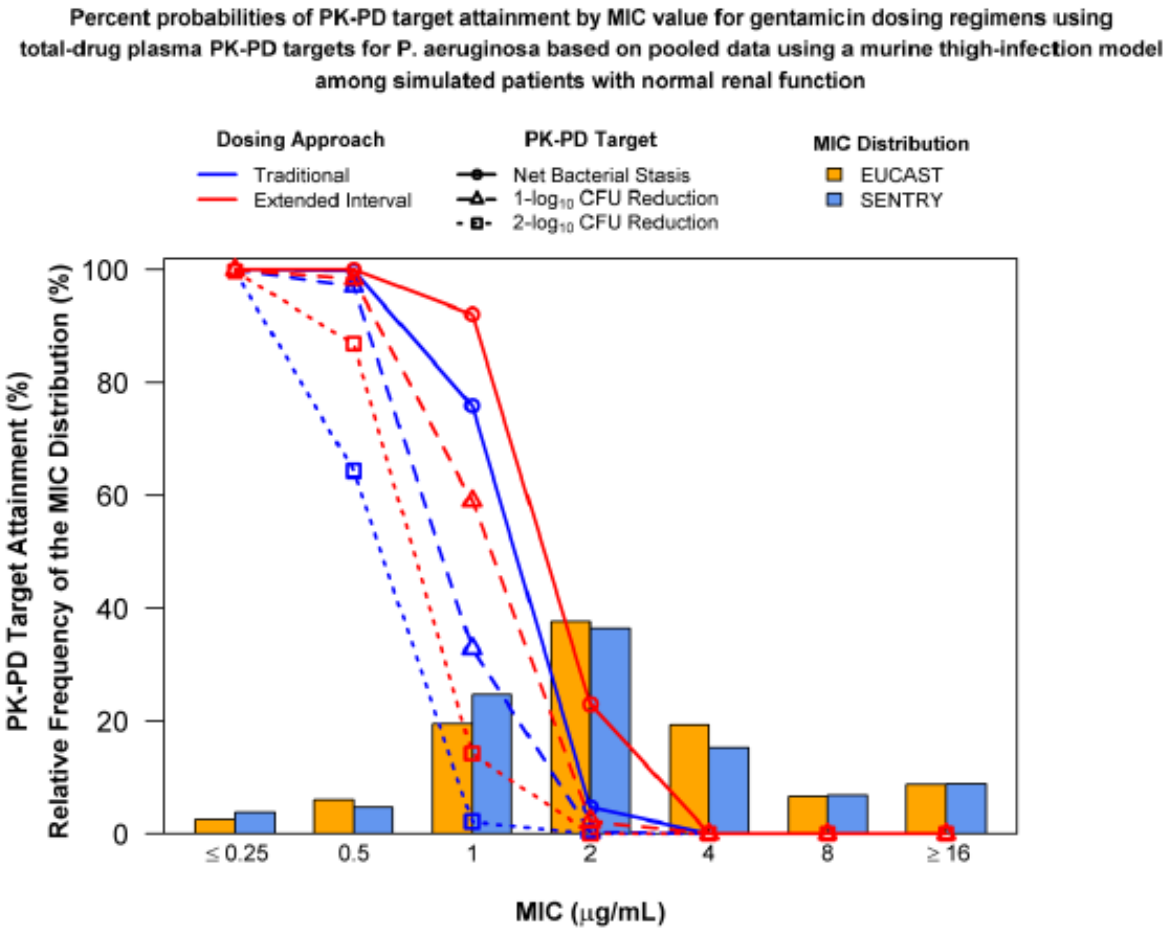
Probability of target attainment (PTA)

>90% PTA is often considered acceptable



Roberts JA et al. J Antimicrob Chemother 2010; 66: 227.

What if the nonclinical PK-PD cutoff falls within the wild-type MIC distribution?



Percent probabilities of PK-PD target attainment by MIC are shown overlaid over MIC distributions from the SENTRY Antimicrobial Surveillance Program (2011-2016, USA) and EUCAST data (2017).

Unlikely to achieve target attainment with any of the gentamicin dosing regimens

This type of modern PK-PD analysis led CLSI to eliminate the gentamicin breakpoint for *Pseudomonas aeruginosa* in 2023

USCAST. Aminoglycoside in vitro susceptibility testing interpretive criteria evaluations. Version 1.3, 2019.

Category #3: Clinical Data

Is there a correlation between MIC and clinical outcome?

Look at a clinical dataset through the lens of outcome-by-MIC:

MIC, µg/mL	Clinical Success
≤ 0.5	4/4 (100%)
1	66/67 (98.5%)
2	102/119 (85.7%)
4	25/38 (65.8%)
8	5/15 (33.3%)
16	1/4 (25.0%)
32	2/15 (13.3%)
[illegible]	[illegible]

Clinical data don't always help us know where the breakpoint should be

- REPROVE
- Non-inferiority trial
- Ceftazidime-avibactam vs. meropenem as definitive therapy for patients with nosocomial pneumonia, including ventilator-associated pneumonia
- Primary endpoint: clinical cure at the test-of-cure visit (21-25 days after randomization)
- Ceftazidime-avibactam was non-inferior

CAZ-AVI MIC_{90} for trial isolates	CAZ-AVI MIC_{90} for isolates we typically treat with CAZ-AVI*
0.5 µg/mL	4 µg/mL

*based on MICs among KPC-producing *K. pneumoniae* in the Pfizer ATLAS database

Torres A et al. Ceftazidime-avibactam versus meropenem in nosocomial pneumonia, including ventilator-associated pneumonia (REPROVE): a randomized, double-blind, phase 3 non-inferiority trial. Lancet Infect Dis 2018; 18: 285.
https://atlas-surveillance.com/

Barriers to determining the clinical cutoff from clinical trials

- Most enrolled patients have highly susceptible isolates → not possible to see a relationship between MIC and outcome
- Identification of the major infecting pathogen may not be straightforward
- Other factors (e.g., host immune status, use of adjunctive treatments) importantly contribute to between-patient variability
- The infections studied don't reflect how the drug will be used in clinical practice

- Ideal datasets would include:
  - Patients with the type of infection for which the drug will be used clinically
  - Clear microbiological diagnoses/monomicrobial infections
  - Reference broth microdilution AST data
  - Organism MICs straddling where you think the breakpoint might be
  - Patients that received a specific dose of drug and had PK studies

- Instead, we are often evaluating clinical data from observational studies performed after a drug comes into use (many caveats…)

Putting it all together

Balancing the three different types of data through a consensus process

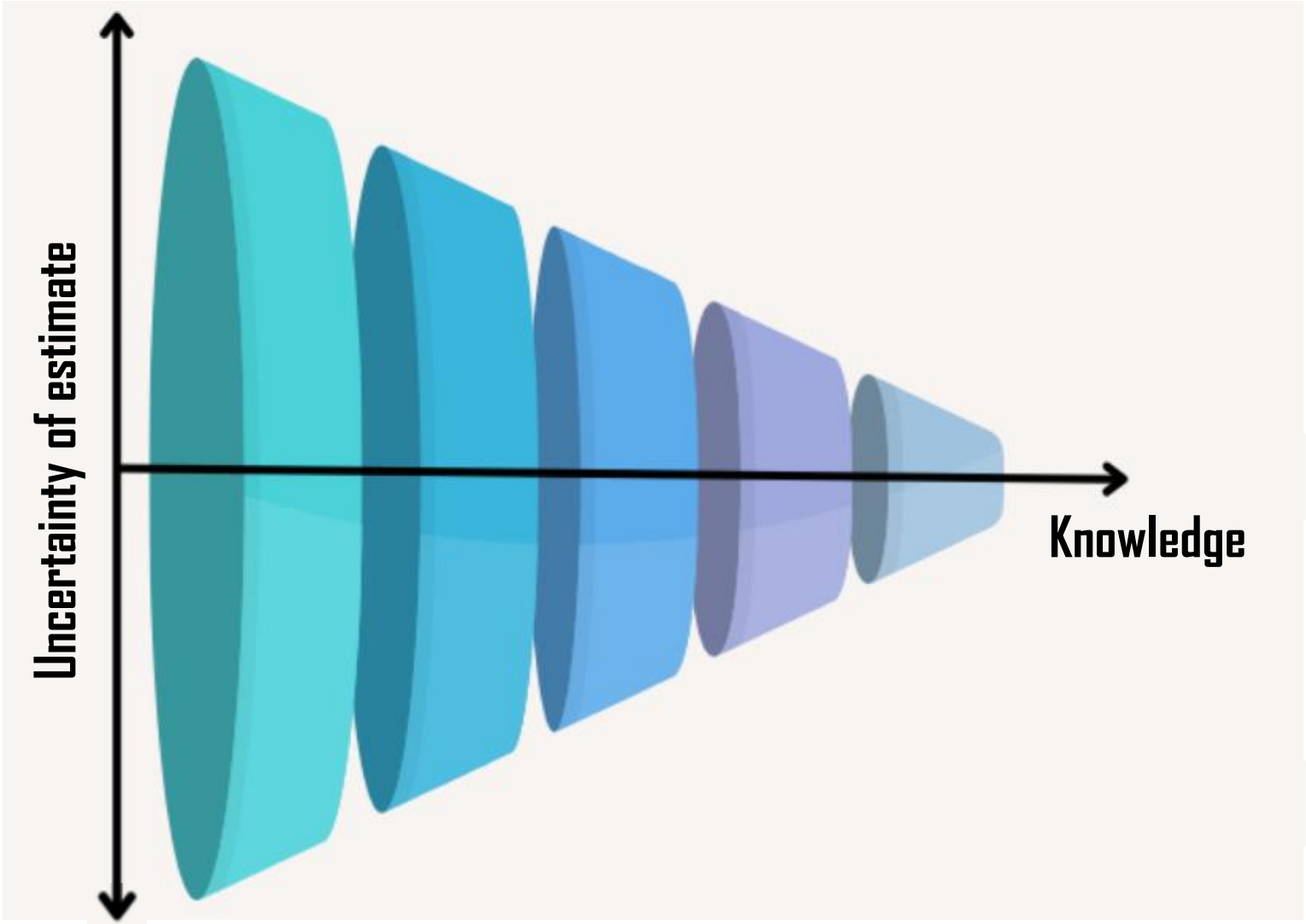
Microbiology	PK-PD		Clinical
Epidemiological cutoff value (ECV)	**Nonclinical PK-PD cutoff**	**Clinical exposure response cutoff**	**Clinical cutoff**
A	B	C	D

Decisions are not formulaic or "one-size-fits-all"

The strengths and limitations of each type of data are weighed in an open consensus-based process involving experts in each type of data and balanced representation from a variety of interested parties (professions, government, and industry)

Question #3:
Why do breakpoints change?

Science leads to increasingly secure knowledge

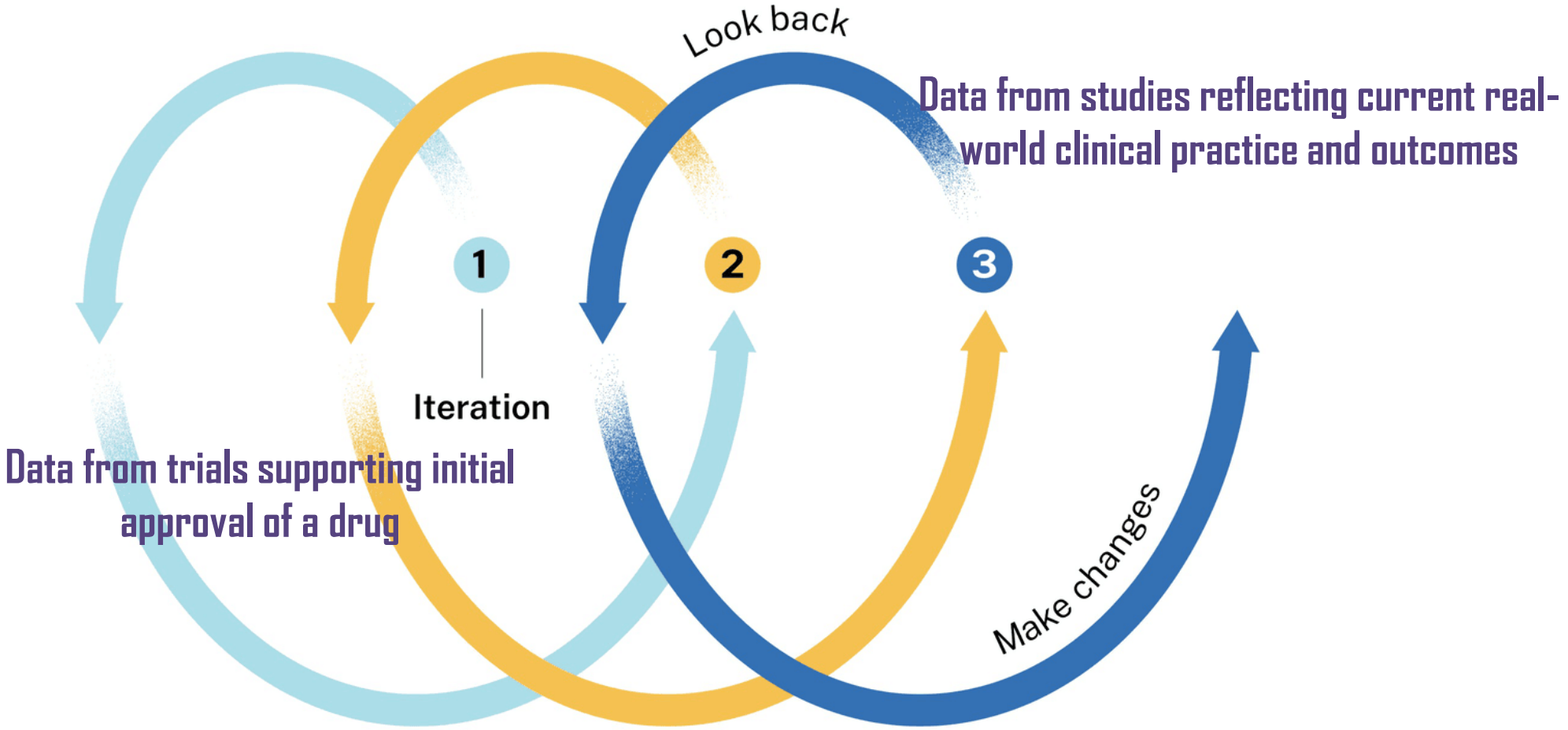


As new data come to light, our understanding evolves and becomes progressively more robust.

This is how science is supposed to work!

Modified from https://scrum-master.org/en/understanding-the-cone-of-uncertainty-and-agile-estimation-mastering-uncertainty-in-your-projects/

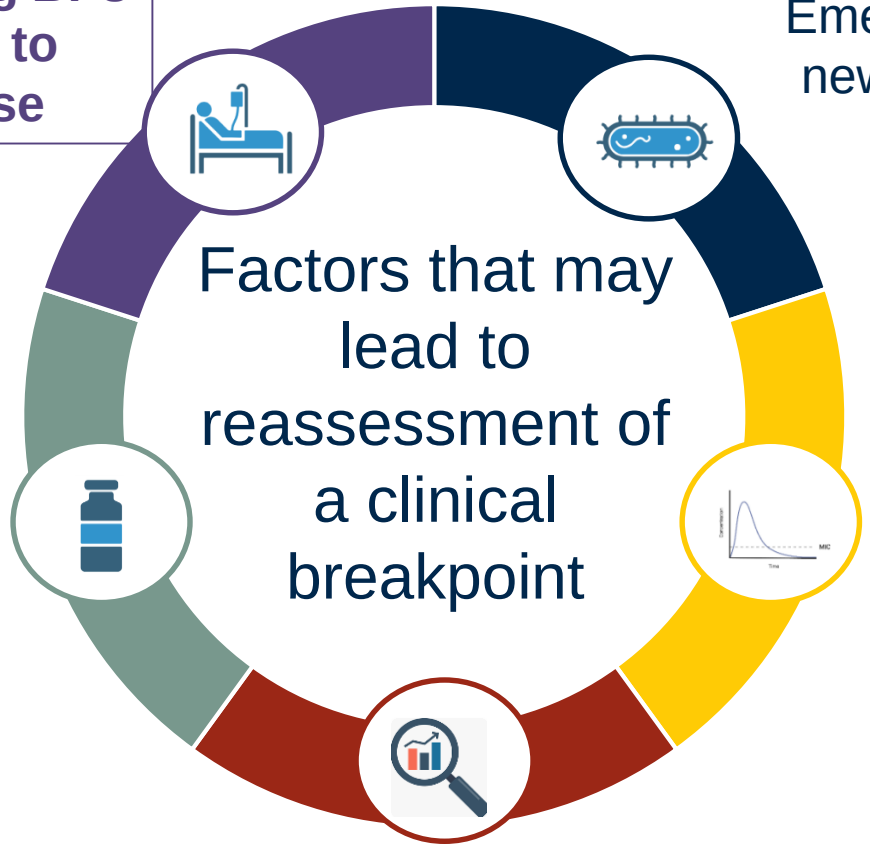
Setting breakpoints is an iterative process



Modified from https://digital.gov/guides/hcd/design-concepts/iteration/

Clinical signal that existing BPs are not performing well to predict clinical response

Emergence (or recognition) of new resistance mechanisms



Prevailing dosage regimens differ substantially from the dosage regimens that were used to establish initial BPs

New PK-PD data indicate that existing BPs may have been set inappropriately high or low

Existing BPs were set before the introduction of current analytical methods used to determine relationships among drug exposure, organism susceptibility, and clinical response

CLSI M23-Ed6; 2023. Icons from biorender.com

Real-world example: the MERINO trial

- Randomized controlled non-inferiority trial
- Piperacillin-tazobactam (TZP) vs. meropenem (MEM) as definitive therapy for patients with ceftriaxone-resistant *E. coli* or *K. pneumoniae* bacteremia
- **Exclusion criteria:** polymicrobial bacteremia, concomitant antibiotics with gram-negative activity, **TZP or MEM resistance (based on local testing)**
- Primary outcome: all cause mortality at 30 days after randomization

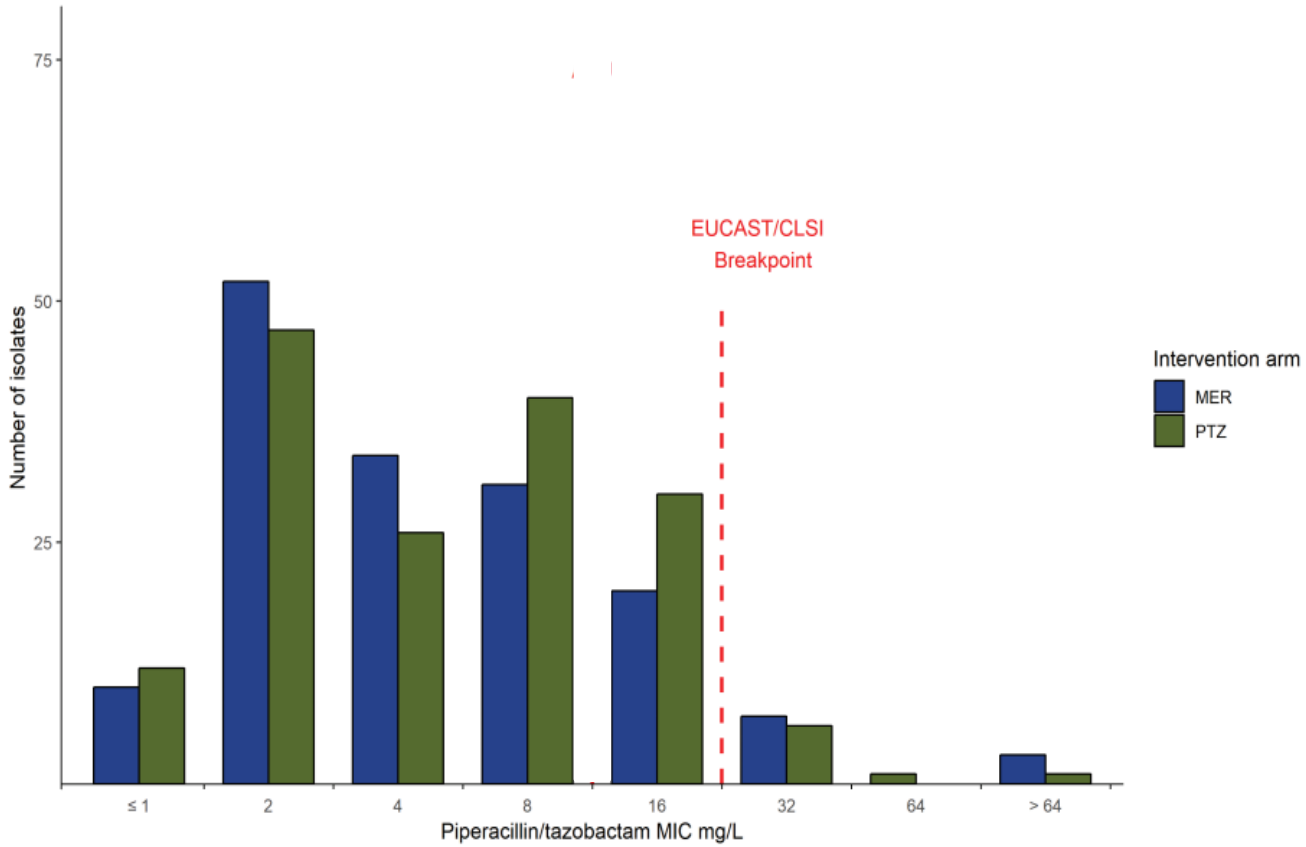
Trial was stopped **early** as a difference in primary outcome was observed at a pre-specified stopping rule (p=0.004)

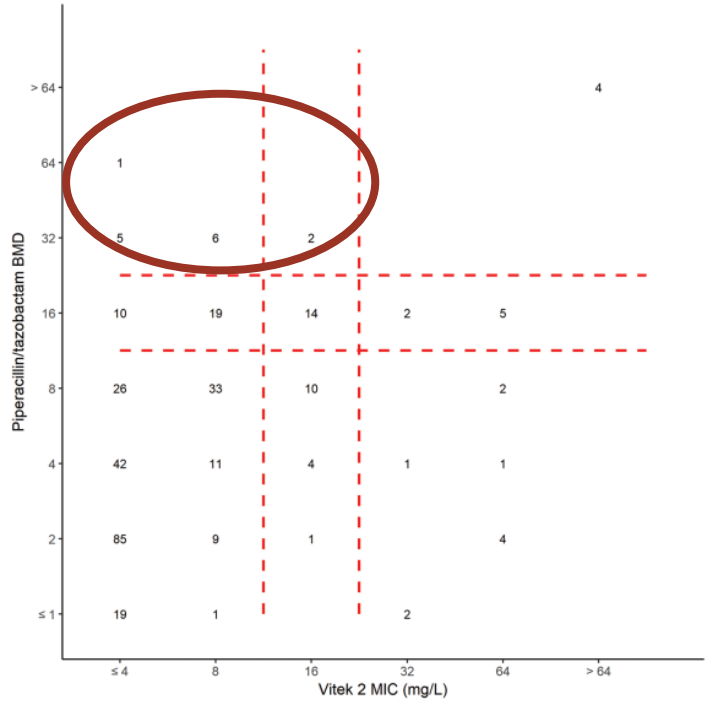
Table 2. Primary Analysis and Subgroup Analyses

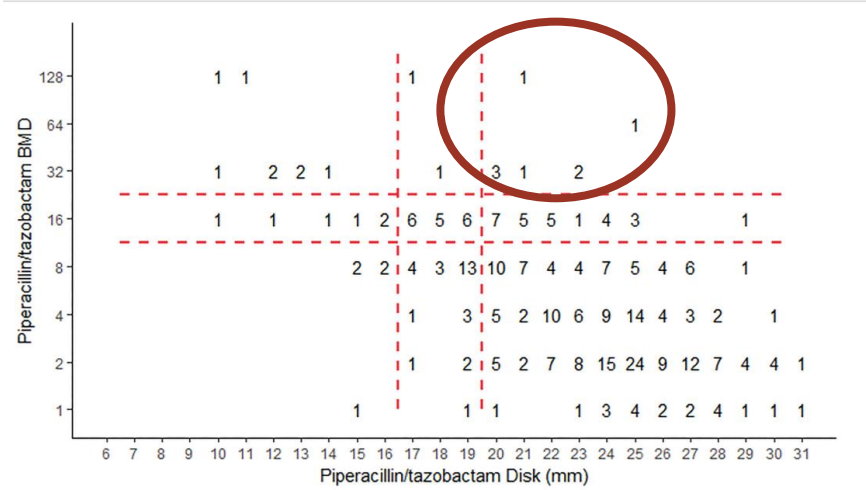
	30-d Mortality, No./Total No. (%)		Risk Difference, % (1-Sided 97.5% CI)[a]	*P* Value for Noninferiority
	Piperacillin-Tazobactam	Meropenem		
Primary analysis	23/187 (12.3)	7/191 (3.7)	8.6 (−∞ to 14.5)	.90
Per-protocol analysis	18/170 (10.6)	7/186 (3.8)	6.8 (−∞ to 12.8)	.76

Harris PNA et al. Effect of piperacillin-tazobactam vs meropenem on 30-day mortality for patients with E. coli or Klebsiella pneumoniae bloodstream infection and ceftriaxone resistance: a randomized clinical trial. JAMA 2018; 320: 984.

Unfortunately, some patients with piperacillin-tazobactam resistant isolates were enrolled

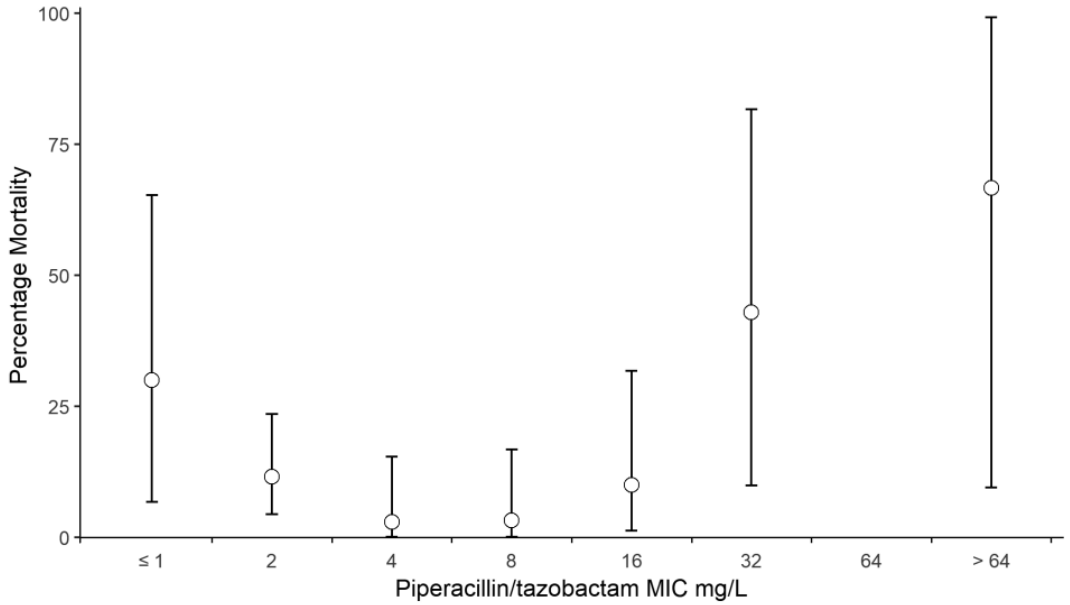






Henderson A et al. Association between minimum inhibitory concentration, beta-lactamase genes and mortality for patients treated with piperacillin-tazobactam or meropenem from the MERINO study. Clin Infect Dis 2021; 73: e3842.

Association between TZP MIC and mortality



	Bivariate Analysis		Multivariate Analysis	
Variable	OR	*P*	aOR	*P*
Log_2(MIC)	1.2 (0.9–1.6)	.20	…	
→ MIC > 16 mg/L	10.3 (2.6–41.9)	<.001	14.9 (2.8–87.2)	.002
UTI source	0.4 (0.2–1.1)	.09	0.6 (0.2–1.8)	.3
Charlson comorbidity score	1.6 (1.3–2.0)[a]	<.001	1.7 (1.3–2.2)[a]	<.001

Abbreviations: aOR, adjusted odds ratio; MIC, minimum inhibitory concentration; UTI, urinary tract infection.

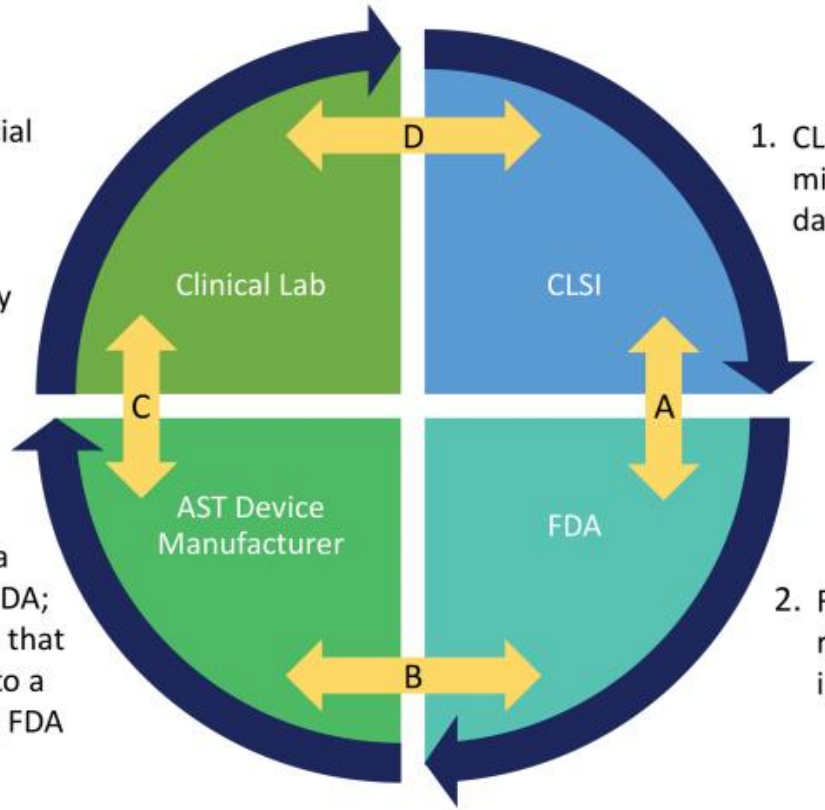
[a]Calculated for each numerical increase in Charlson Comorbidity Score.

Important driver of a comprehensive review of the Enterobacterales piperacillin-tazobactam breakpoint by CLSI
→ lowered breakpoint in 2022

Henderson A et al. Association between minimum inhibitory concentration, beta-lactamase genes and mortality for patients treated with piperacillin-tazobactam or meropenem from the MERINO study. Clin Infect Dis 2021; 73: e3842.

Breakpoints exist within a life cycle

4. Laboratories frequently use commercial AST devices and rely on device manufacturers to use up-to-date FDA breakpoints; labs can also use a CLSI breakpoint if its performance is locally validated



1. CLSI reviews the available microbiological, PK-PD, and clinical data and sets a breakpoint

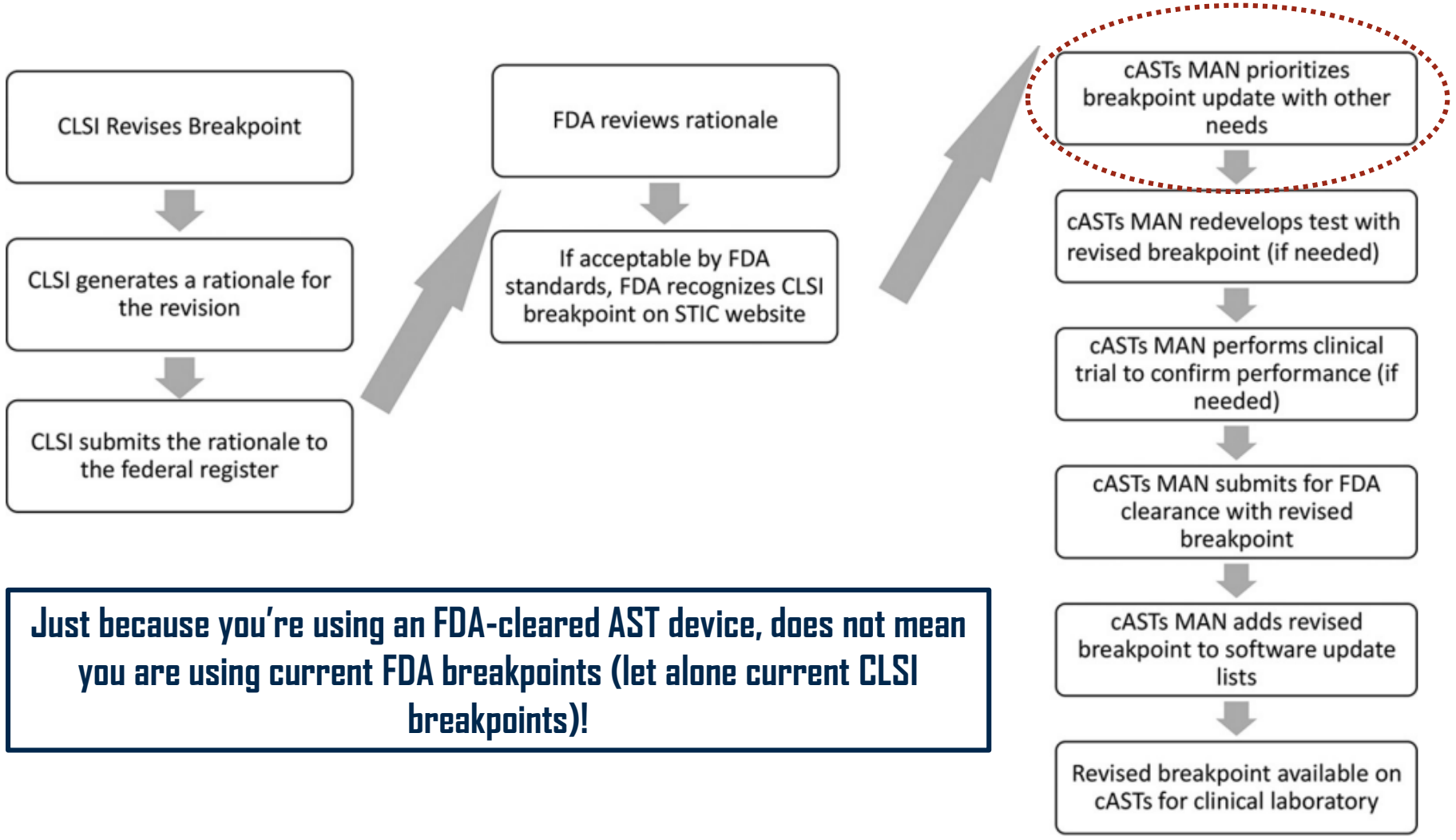
3. AST device manufacturer can adopt a new breakpoint when approved by FDA; the manufacturer must demonstrate that the device performs well compared to a reference AST method when seeking FDA clearance

2. FDA reviews CLSI rationale and adopts or rejects the CLSI breakpoint; FDA can also independently set a breakpoint

A. Not all breakpoints are reviewed by CLSI. Data from a sponsor (typically, the drug manufacturer) is generally critical for review, and some sponsors may bring data for review to FDA but not to CLSI.	B. FDA recognizes breakpoints for certain organism-antimicrobial combinations. AST device manufacturers are then bound to focus on these breakpoints for FDA clearance.
C. Commercial AST devices cleared to use FDA breakpoints are installed in clinical laboratories. Clinical labs can validate other breakpoints, including using commercial AST devices, if the capabilities of the commercial AST device allow. AST device manufacturers are motivated by market forces to have up-to-date breakpoints.	D. Clinical labs use breakpoints set by CLSI. When evidence emerges of failure of a current breakpoint, often from signals from clinical labs and clinicians, CLSI can respond by reviewing the issue and may reconsider the breakpoint.

Pierce VM, Mathers AJ. J Pediatr Infect Dis Soc 2022; 11: 73.

FDA cleared device ≠ current breakpoints!



Just because you're using an FDA-cleared AST device, does not mean you are using current FDA breakpoints (let alone current CLSI breakpoints)!

Manufacturers are not required to update BPs after their devices have received FDA clearance; they can continue to market "legacy" devices.

Market forces motivate decisions about whether to pursue clearance with updated FDA BPs.

FDA is working to make it easier for manufacturers to update BPs.

Humphries RM et al. J Clin Micro 2019; 57: e00203. https://www.fda.gov/media/172463/download (guidance issued 9/2923)

Question #4:
Why should labs use current breakpoints?

How are our AST results being utilized?

1 **Predict clinical outcome**

Guide targeted antimicrobial therapy in individual patients

Each of these applications are impacted by using an outdated breakpoint

Guide targeted antimicrobial therapy in individual patients

Carbapenems are prescribed for treatment of CRE (including CPO) infections, leading to bad patient outcomes

Aid infection prevention

Patients with CPOs go unrecognized, allowing carbapenemases to spread from patient to patient across the healthcare system and the community

Example:
Labs are using outdated (too high) carbapenem breakpoints for Enterobacterales

Inform empiric therapy

Institutional rates of CRE and CPOs are underestimated when developing treatment guidelines and when making formulary decisions

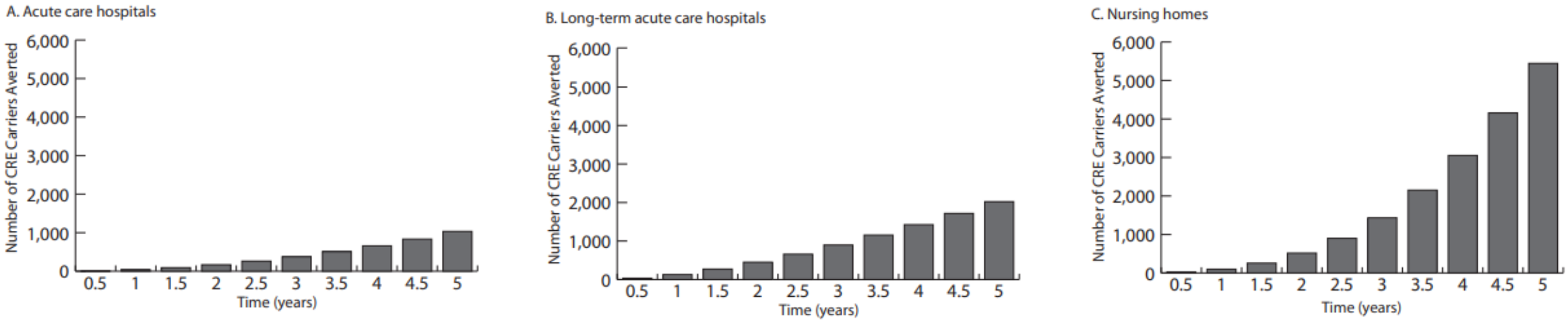
Track resistance

Underreporting leads to inaccurate understanding of the current scope of the problem and reduced ability to measure the impact of interventions

Orange County example

Used a simulation to model the impact of a delay in implementing updated carbapenem breakpoints on the number of CRE carriers in a single county in California

Even though the new (lower) breakpoints identified more existing CRE carriers, their identification resulted in fewer cases of transmission due to the use of contact precautions

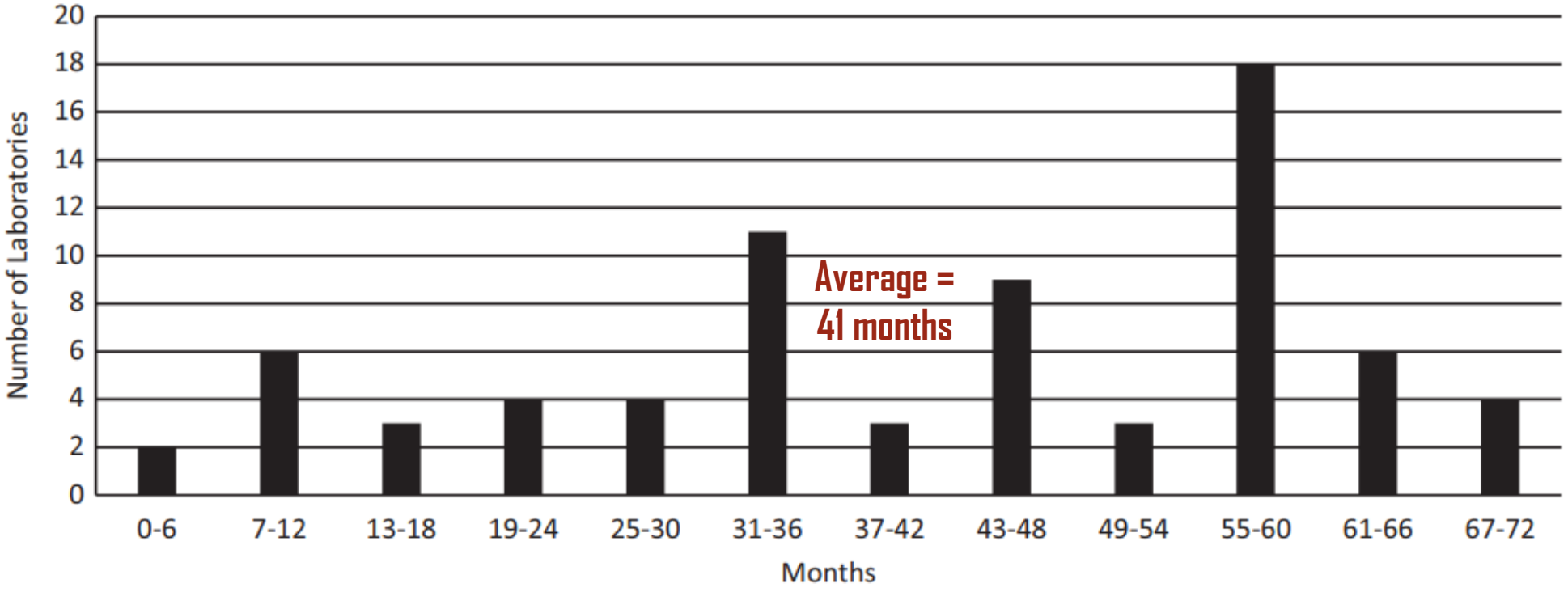


2.5-year delay in implementing new breakpoints would have resulted in **~1,821 more CRE carriers** countywide

Bartsch SM et al. J Clin Microbiol 2016; 54: 2757.

Delays are (or at least, have been) a reality

Timeline to implementation of current carbapenem breakpoints for Enterobacterales in California (among the 74 labs of 128 surveyed that disclosed this information)



Humphries RM et al. Clin Infect Dis 2018; 66: 1061.

Outdated breakpoint use common among CAP-accredited US laboratories in 2019

Organism	Antimicrobial Agent	United States	
		Total No. of Laboratories	Current Break-points, No. (%)
Enterobacterales	Ceftazidime	1046	620 (59.3)
Enterobacterales	Ceftriaxone	1124	694 (61.7)
Enterobacterales	Ciprofloxacin	1058	312 (29.5)
Enterobacterales	Levofloxacin	1019	306 (30.0)
Enterobacterales	Meropenem	982	610 (62.1)
Pseudomonas aeruginosa	Piperacillin-tazobactam	1064	559 (52.5)
Acinetobacter baumannii	Imipenem	784	367 (46.8)

Depending on the bug-drug combination, **37.9-70.5%** of labs reported using obsolete interpretive criteria

Simner PJ et al. Open Forum Infect Dis 2022; 9: ofac007.

Why were labs using obsolete breakpoints?

Reason	United States (n = 835)
Efforts to use or implement current breakpoints underway	372 (44.6)
Plan to update, in progress	181 (48.7)
Not applicable because do not report, use alternate method, or send to reference laboratory	102 (27.4)
Changing panels or instruments	55 (14.8)
Validation testing not completed but underway	34 (9.1)
Ongoing use of obsolete breakpoints, no current revisions in progress	463 (55.4)
Manufacturer-related issues	232 (50.1)
Resource limitations of staff, time, organisms, guidance, laboratory information system issues, cost	112 (24.2)
Overlooked or unaware of breakpoint change or need to update	57 (12.3)
Facility does not support	30 (6.5)
Not done, under review for a variety of concerns	28 (6.0)
Do not want or intend to update	4 (0.8)

Data are presented as No. (%).

Simner PJ et al. Open Forum Infect Dis 2022; 9: ofac007.

CAP checklist update put labs in the hot seat!

At minimum, CAP-accredited labs need to implement updated FDA BPs within 3 years of publication, even if their AST device still uses obsolete BPs.

Labs can also implement CLSI BPs, even those not recognized by FDA.

Both scenarios constitute modifications of the device's IFU that require **validation**.

MIC.11385 **Current Antimicrobial Susceptibility Test Interpretation Breakpoints** **Phase I**

Effective January 1, 2024, the laboratory uses current breakpoints for interpretation of antimicrobial minimum inhibitory concentration (MIC) and disk diffusion test results. New breakpoints are implemented within three years of the date of publication by the FDA for laboratories subject to US regulations, or within three years of publication by CLSI, EUCAST or other standards development organization (SDO) for laboratories not subject to US regulations.

NOTE 1: For laboratories subject to US regulations, a breakpoint is considered obsolete three years after publication of an update by the FDA, though the laboratory may use currently accepted breakpoints from other SDOs with validation to support use. SDOs that develop breakpoints include the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Whether using breakpoints from the FDA or other SDOs, US laboratories must, at a minimum, adopt the change within three years of the official publication date of the updated breakpoint by the FDA.

● ● ●

Evidence of Compliance:

- ✓ Records of validation reports for breakpoints that differ from those included in the FDA-clearance of an instrument **AND**
- ✓ Records of the interpretive criteria used for antimicrobial susceptibility testing **AND**
- ✓ Source document (including year of publication) from which the interpretive criteria were derived **AND**
- ✓ Patient or LIS reports with interpretations matching the source document

CAP Accreditation Program Microbiology Checklist, December 2024.

Labs have been putting in the work

- Labs may be able to use up-to-date breakpoints with their existing commercial AST device if it includes the appropriate dilutions, following performance validation
- Resources with expert guidance:

2023 Breakpoint Implementation Toolkit

- Archived CLSI-CAP webinar (Breakpoints Matter)
- Archived CLSI BIT webinar (Get Current)
- CLSI M68 document forthcoming in 2026

https://clsi.org/meetings/ast/breakpoints-in-use-toolkit/

What do these validations realistically look like?

- Unreasonable to expect individual clinical laboratories to truly **establish** performance specifications for an AST when using off-label breakpoints in the same way that a commercial device manufacturer would be expected to do (large clinical trials)
- In some cases, breakpoint update validations may consist of reanalyzing existing data; in others, labs may need to test some contemporary isolates, but will not have resources to test huge numbers
- Lab directors may take a risk-based approach, weighing the risks of **not** updating the breakpoints vs. small challenges identified with testing (i.e., do I care more about a few minor errors or about the % of my isolate population that tests "S" by the old breakpoints but "R" by the new breakpoints?)
- Labs that do have the resources for larger studies looking at the performance of commercial AST devices with updated breakpoints should consider doing those studies and publishing their results to help inform decision-making across the clinical microbiology community

Question #5:

How would the FDA's new LDT rule create a Catch-22 for labs?

FDA Laboratory-Developed Tests (LDT) Rule

- FDA released their proposed oversight rule on 9/26/23 and their final rule (500 pages!) on 4/29/24 (officially published on 5/6/24)
- Rule says that FDA will start regulating tests (or in their words, "phase out enforcement discretion") when the manufacturer of a test is a laboratory
  - i.e., they consider LDTs "devices" under the Federal Food, Drug & Cosmetic Act

But wait! Does modifying the breakpoints really turn my FDA-cleared AST into an LDT?

- Yes. Using breakpoints that are different than those for which a device received FDA clearance is considered by FDA to constitute a "significant modification that could affect the safety or effectiveness of the test"

- This is true even if the breakpoints you want to use are those currently recognized by FDA. If the manufacturer has not sought and received clearance of their device with the updated breakpoints, updating them in an individual lab → LDT

Example: Sensititre Gram-Negative GN7F AST Plate – *Pseudomonas aeruginosa* (as of 5/6/24)

Drug	Device clearance	Current CLSI	Current FDA
Piperacillin-tazobactam	≤ 64/4 S, ≥ 128/4 R	≤ 16/4 S, 32/4 I, ≥ 64/4 R	M100 recognized
Ceftazidime	≤ 8 S, 16 I, ≥ 32 R*	≤ 8 S, 16 I, ≥ 32 R	≤ 8 S, ≥ 16 R
Cefepime	≤ 8 S, 16 I, ≥ 32 R*	≤ 8 S, 16 I, ≥ 32 R	≤ 8 S, ≥ 16 R
Ceftazidime-avibactam	≤ 8/4 S, ≥ 16/4 R	≤ 8/4 S, ≥ 16/4 R	M100 recognized
Ceftolozane-tazobactam	≤ 4/4 S, 8/4 I, ≥ 16/4 R	≤ 4/4 S, 8/4 I, ≥ 16/4 R	M100 recognized
Aztreonam	≤ 8 S, 16 I, ≥ 32 R*	≤ 8 S, 16 I, ≥ 32 R	M100 recognized
Imipenem	≤ 4 S, 8 I, ≥ 16 R*	≤ 2 S, 4 I, ≥ 8 R	M100 recognized
Meropenem	≤ 4 S, 8 I, ≥ 16 R*	≤ 2 S, 4 I, ≥ 8 R	M100 recognized
Ciprofloxacin	≤ 1 S, 2 I, ≥ 4 R*	≤ 0.5 S, 1 I, ≥ 2 R	M100 recognized
Levofloxacin	≤ 2 S, 4 I, ≥ 8 R*	≤ 1 S, 2 I, ≥ 4 R	M100 recognized
Tobramycin	≤ 4 S, 8 I, ≥ 16 R*	≤ 1 S, 2 I, ≥ 4 R	≤ 4 S, 8 I, ≥ 16 R

*No breakpoint listed in the *Pseudomonas aeruginosa* only column of IFU; breakpoint listed pulled from "non-Enterobacteriaceae" column of IFU

On-label use; validating these BPs = LDT; impossible under LDT rule since FDA only clears devices that use FDA breakpoints

Why are manufacturers reluctant to submit devices for clearance with current FDA breakpoints?

- May require submission of new data to FDA (time and $$$)
- Risk of losing other claims, for example:
  - Sensititre meropenem was cleared with generic "non-Enterobacteriaceae" breakpoints of ≤ 4, 8 I, ≥ 16 R many years ago
  - CLSI subsequently set a different meropenem breakpoint for *P. aeruginosa* (≤ 2, 4 I, ≥ 8 R), and this was recognized by FDA
  - If Sensititre goes to FDA with data showing that their meropenem test works well with the updated *P. aeruginosa* breakpoints, FDA will review meropenem performance for all organisms tested with the device
  - Since FDA only clears devices that use FDA breakpoints, Sensititre would lose their grandfathered claim for meropenem testing of "other non-Enterobacterales" (e.g., non-*aeruginosa Pseudomonas, Achromobacter* spp., etc.) because FDA does not have meropenem breakpoints for these organisms

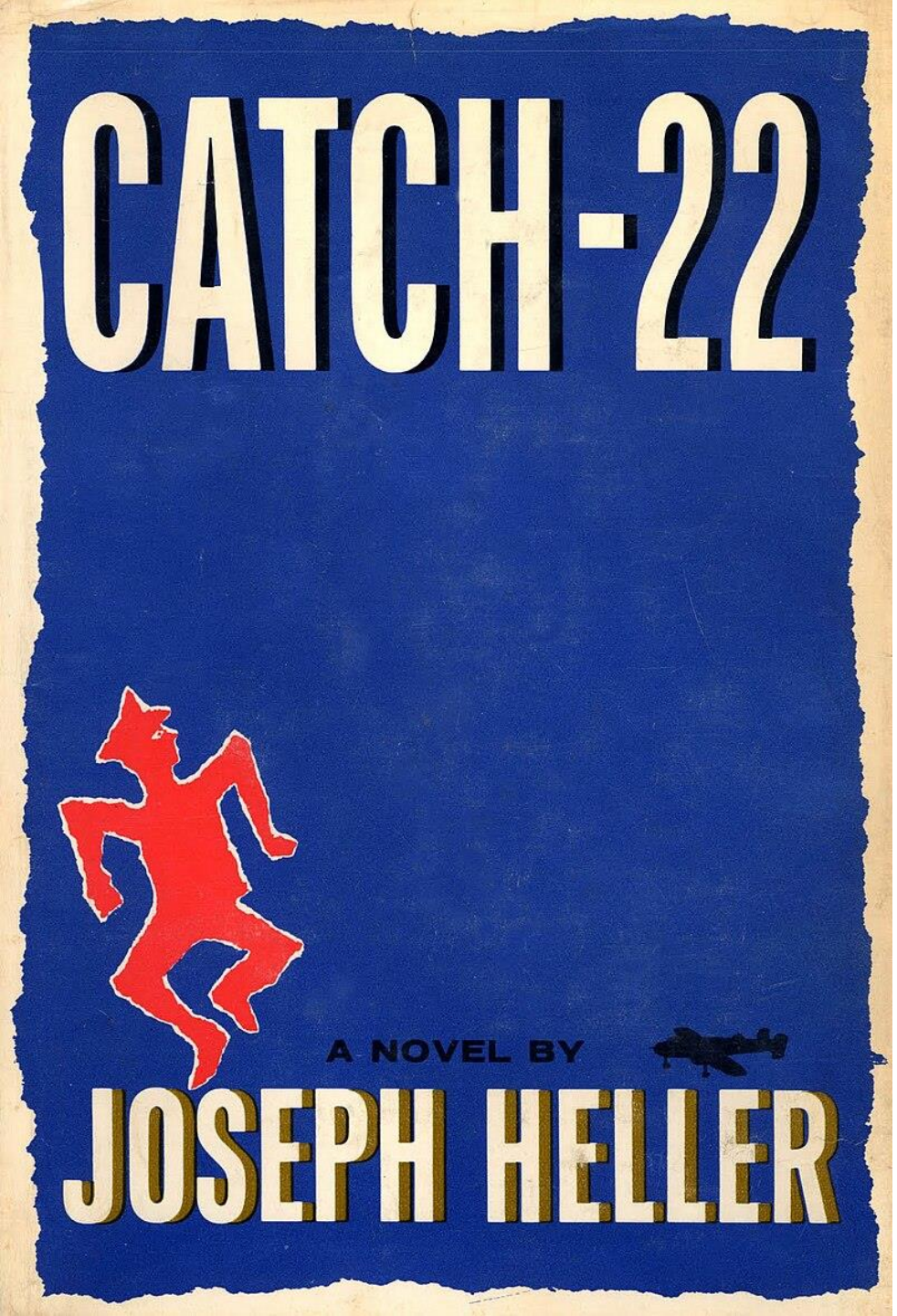
“Legacy” device cleared with now obsolete breakpoints

Use on-label with obsolete breakpoints, risking patient safety and out of compliance with CAP requirements?

Pressure device manufacturer to update to the current FDA breakpoints, risking loss of claims for other organisms?

Validate as an LDT with the current FDA breakpoints?

Stop testing this bug-drug combination with this device and bring on a new system that can be used on-label?



A problem for which the only solution is denied by a circumstance inherent in the problem or by a rule

Merriam-Webster

A tricky problem; a no-win or absurd situation

Wikipedia

CAP requires up-to-date breakpoints (good for patients)

Our primary solution to breakpoint gaps has been modification of commercial AST devices for off-label use

But now labs would be put into an impossible situation by the FDA's LDT Rule

Most labs wouldn't have the resources to do everything required under the rule

Some AST would become impossible, since in the absence of an FDA breakpoint, FDA will not authorize a test

Clinical Infectious Diseases

VIEWPOINTS

How New Regulation of Laboratory-Developed Antimicrobial Susceptibility Tests Will Affect Infectious Diseases Clinical Practice

Kaleb H. Wolfe,[1] Virginia M. Pierce,[2] and Romney M. Humphries[3]

[1]Department of Infectious Diseases, Vanderbilt University Medical Center, Nashville, Tennessee; [2]Department of Pathology, University of Michigan Medical School, Ann Arbor; and [3]Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, Tennessee

At the time of publication (April 2024), there were >220 differences between CLSI and FDA breakpoints, 173 of which were situations in which CLSI had a breakpoint published in the M100 that FDA did not recognize and for which FDA had no breakpoint

This tally did not include any of the breakpoints in the CLSI M45 document (e.g., *Abiotrophia*, *Aeromonas*, etc.)

Clinical vignettes describing what would happen under the FDA LDT Rule given the lack of FDA breakpoints for bug-drug combinations like daptomycin with *Enterococcus faecium*, TMP-SMX and doxycycline with *Staphylococcus aureus*, and any drugs with *Stenotrophomonas maltophilia* – these are not esoteric scenarios!

Wolfe KH et al. Clin Infect Dis 2024; 78: 1140.


THIS IS
FINE

Exemptions from pre-market review

- Tests first marketed before 5/6/2024
  - Not exempt from compliance with first two phases, including listing and labeling
  - All bets are off if you modify something important (for example, a breakpoint)

https://www.fda.gov/medical-devices/laboratory-developed-tests-faqs/phaseout-policy-and-enforcement-discretion-policies-laboratory-developed-tests-faqs

Exemptions from pre-market review

- "Unmet need" LDTs
  - Manufactured and performed by a laboratory integrated within a healthcare system to meet an unmet need for patients within the same healthcare facility
    - Does not include patients being treated at an affiliated hospital with a different corporate ownership than the laboratory
    - Limited to LDTs that are ordered by a healthcare practitioner on the staff or with credentials and privileges at a facility owned and operated by the same healthcare system employing the laboratory director and performing the LDT (FDA believes the shared responsibility and potential liability for patient outcomes mitigates risk)
  - "Unmet need" means there is no available FDA-authorized IVD that meets the patient's needs
  - The decision-making process for determining if an LDT qualifies for the "unmet need" exemption was not clarified in the FDA rule and had remained unclear

https://www.fda.gov/medical-devices/laboratory-developed-tests-faqs/phaseout-policy-and-enforcement-discretion-policies-laboratory-developed-tests-faqs

What counts as an "unmet need"?

- There is no FDA-cleared AST for a bug-drug combination because there is no FDA breakpoint, and so FDA clearance is not possible?
- There is no FDA-cleared AST for a bug-drug combination for which there is an FDA breakpoint, but for which no commercial manufacturer has (yet) sought clearance?
- There is no AST that was cleared with the current FDA breakpoints for a bug-drug combination, only ASTs cleared with obsolete breakpoints?
- There is at least one FDA-cleared AST device for the bug-drug combination that uses current FDA breakpoints, but my lab doesn't own the necessary instrumentation?
- There are FDA-cleared AST devices for the bug-drug combination, but CLSI breakpoints differ from FDA breakpoints? Probably not, since FDA states that "potential improvement in performance" does not fall within this policy…?

Question #6:
How do we get out of this mess?!?

Deus ex machina

"god from the machine" – a plot device whereby a seemingly unsolvable problem in a story is suddenly or abruptly resolved by an unexpected and unlikely occurrence

Court Throws Out LDT Rule

Release Date: 31 Mar 2025

https://www.quora.com/How-many-Greek-plays-actually-employed-Deus-Ex-Machina
https://newsroom.cap.org/latest-news/court-throws-out-ldt-rule/s/3f27177c-4473-401a-95cc-eefdbefa64fa

https://www.linkedin.com/pulse/traversing-twists-turns-guide-finding-clear-path-forward-shaik-saleem/

Potential paths forward if LDT Rule stood?

- **Path #1: AST carve-out**
  - FDA could create a carve-out for AST from LDT regulation (keep the status quo)
  - Downside of this approach is that the status quo is not great – we have lots of breakpoint gaps and the burden is on clinical labs to close those gaps to deliver the highest quality patient care

Wolfe KH et al. Clin Infect Dis 2024; 78: 1140.

Potential paths forward if LDT Rule stood?

- **Path #2: "MIC only" AST device clearance**
  - FDA could move to a system whereby they clear AST devices on an "MIC only" basis (i.e., focus on essential agreement and bias as performance criteria, rather than categorical agreement)
  - Would align with the ex-US approach, where ISO 20776-2 guidance is followed to determine the performance of AST devices
  - Would ensure accuracy of test results (MICs) while allowing interpretation of those MICs using the most up-to-date breakpoints according to CLSI
  - Would remove the requirement for commercial device manufacturers to resubmit to FDA when breakpoints are updated, leading to much faster implementation

Wolfe KH et al. Clin Infect Dis 2024; 78: 1140. Patel JB et al. J Clin Microbiol 2023; 61: e0115422. ISO-20776-2; 2021.

Potential paths forward if LDT Rule stood?

- **Path #3: Broad recognition of CLSI breakpoints by FDA**
  - FDA could decide to much more broadly recognize CLSI breakpoints, especially for high priority bug-drug combinations
  - We then need AST device manufacturers to rapidly submit devices for clearance with these newly recognized breakpoints
    - We need FDA to be clearer about the specific data required for breakpoint updates and to streamline the submission pathway
    - The FDA Special Controls Document that gives guidance to AST device manufacturers was last updated in 2009, and yet the expectations have significantly evolved in the interim as evidenced by FDA decisions outlined in 510(k) decision summaries – manufacturers basically have to deduce the unwritten rules through careful examination of FDA's decisions

Wolfe KH et al. Clin Infect Dis 2024; 78: 1140. Patel JB et al. J Clin Microbiol 2023; 61: e0115422. Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems, document issued August 28, 2009.

Updates to FDA's STIC website
1/16/25 and 2/12/25

FDA U.S. FOOD & DRUG ADMINISTRATION

Updates to Standards Recognition

As of February 12, 2025, unless specific exceptions and additions are identified, FDA fully recognizes the standards published in:

- Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 35th ed. CLSI supplement M100; 2025.

As of January 16, 2025, unless specific exceptions and additions are identified, FDA fully recognizes the standards published in:

- Clinical and Laboratory Standards Institute (CLSI). Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria. 3rd ed. CLSI document M45; 2015.

(Similar language about the CLSI yeast, filamentous fungi, mycobacteria, and mycoplasma AST documents!)

Some differences remain, but the list of breakpoint gaps between CLSI and FDA suddenly got a whole lot shorter!

https://www.fda.gov/drugs/development-resources/notices-updates

What should we be doing in the meantime?

- Take inventory of which ASTs in your lab qualify as LDTs (consider using the organizational framework you developed to take stock of your "breakpoints in use" in fulfillment of CAP checklist requirements)
- Make sure your organization is prepared to meet the phase 1 requirements for all LDTs: compliance with medical device reporting (MDR) requirements, correction and removal reporting requirements, and quality system (QS) requirements regarding complaint files
- Stay in touch with your AST device manufacturer(s) about their plans to seek clearance with updated breakpoints given recent updates to FDA STIC
- Continue to work with your antimicrobial stewardship team to prioritize and implement breakpoint updates, using the validation and risk assessment strategies you think are appropriate
- Pay attention to news and information about this topic (including that shared by your professional societies)

https://personal-evolution-company.mykajabi.com/blog/no-sticking-your-head-in-the-sand

Thank you!

vpierce@med.umich.edu

UNIVERSITY OF MICHIGAN
MEDICAL SCHOOL
MICHIGAN MEDICINE

Interpreting Discordant Genotypic and Phenotypic Antimicrobial Susceptibility Testing

Rachael Liesman, PhD, D(ABMM)
Associate Professor
Medical College of Wisconsin
Director, Clinical Microbiology and Molecular Diagnostic Laboratory
Froedtert Hospital / Wisconsin Diagnostic Laboratories
rliesman@mcw.edu

Genotypic vs Phenotypic Antimicrobial Susceptibility Testing

Phenotypic AST

Detection of arrest of bacterial cell growth in the presence of antimicrobial agent
Automated AST instrument, gradient diffusion, disk diffusion, broth microdilution

Genotypic AST

Detection of genes known to correlate with antimicrobial resistance
Currently available genotypic AST

** **Blood Culture ID** **

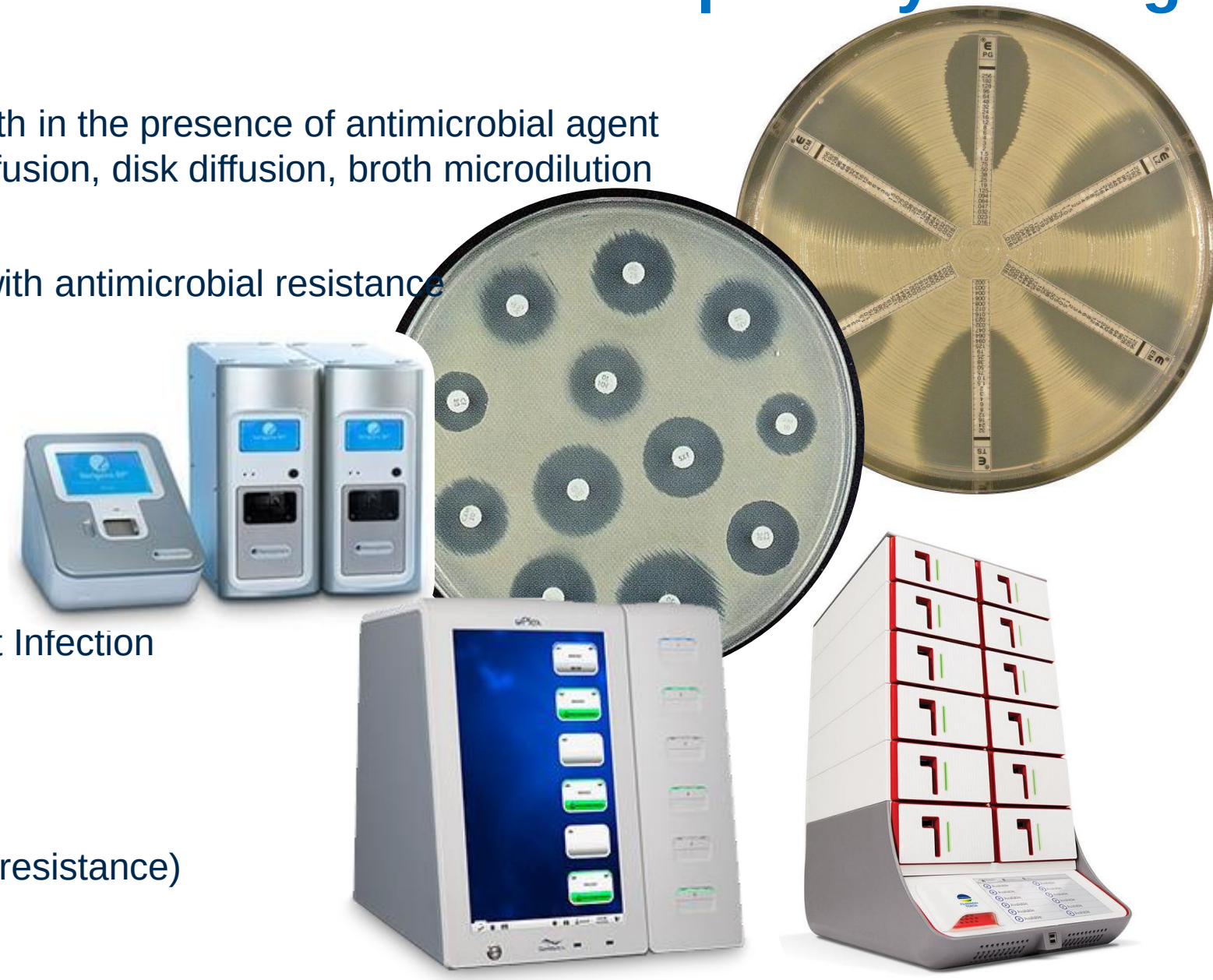
- Luminex Verigene
- BioFire FilmArray
- Roche (GenMark) ePlex

Multiplex Syndromic Panels

BioFire FilmArray Pneumonia, Joint Infection

Narrow/single target tests

MRSA screen (mecA/mecC)
VRE screen (vanA/vanB)
M. tuberculosis complex PCR (RIF resistance)



Genotypic vs Phenotypic Antimicrobial Susceptibility Testing

Resistance Genes	Relevant Organisms	Antimicrobial
Gram Positive Organisms		
mecA *mecC*	*Staphylococcus* species	Oxacillin and/or cefoxitin
vanA *vanB*	*Enterococcus* species	Vancomycin

Genotypic vs Phenotypic Antimicrobial Susceptibility Testing

Resistance Genes	Relevant Organisms	Antimicrobial
Gram Positive Organisms		
mecA *mecC*	*Staphylococcus* species	Oxacillin and/or cefoxitin
vanA *vanB*	*Enterococcus* species	Vancomycin
Gram Negative Organisms		
CTX-M (ESBL)	Enterobacterales	Ceftriaxone, cefotaxime
Carbapenemases KPC NDM VIM IMP OXA23/48	Enterobacterales *P. aeruginosa* *Acinetobacter* species	Ertapenem Meropenem

Limitations

Limited to bug/drug combinations with single (or narrow) mechanism of resistance

mecA detection predicts methicillin-resistant *S. aureus*

vanA/B detection predicts vancomycin resistant *Enterococcus* species

Antimicrobial resistance (AMR) genes not on panel may be missed

mecC detection may be missed if panel detects only *mecA* -> miss MRSA

vanB detection may be missed if panel detects only *vanA* -> miss VRE

Complex mechanisms of resistance lead to lower predictive power

Absence of marker does not necessarily predict susceptibility

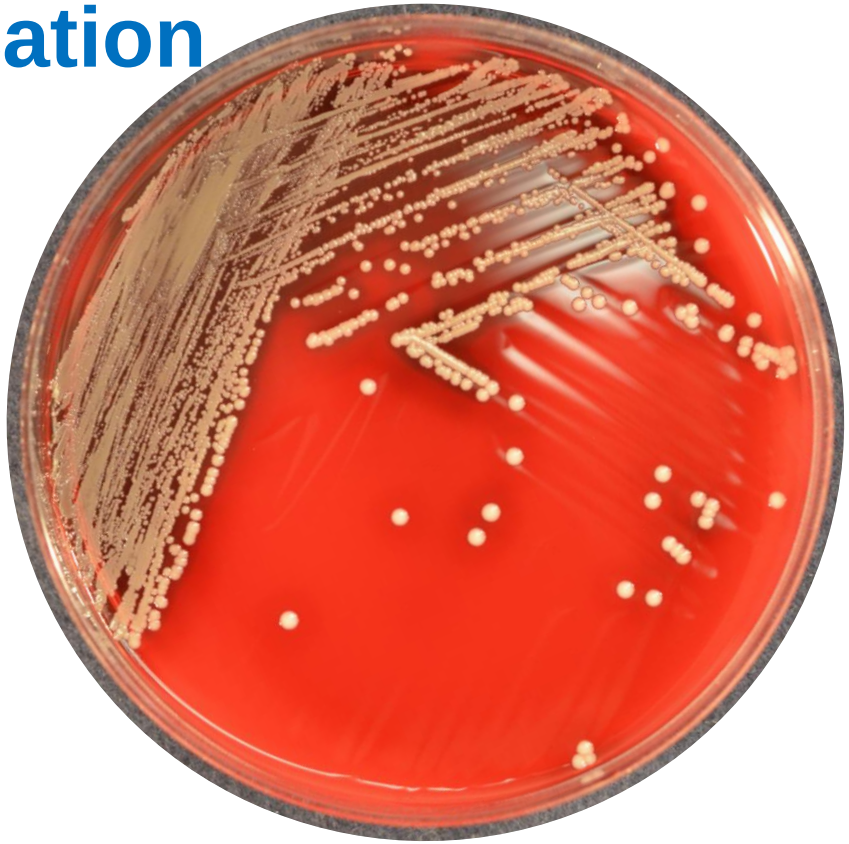
Barrier to prediction in Gram negative organisms

Lack of detection of CTX-M does not predict cephalosporin activity

Lack of detection of carbapenemase genes does not predict carbapenem activity

Genotypic AST is performed in addition to (not in lieu of) phenotypic AST

Implementing Genotypic Susceptibility Testing: Reporting and Communication



Reporting

Incorporate into culture results vs separate line list

Considerations:

LIS/EMR capabilities

Billing

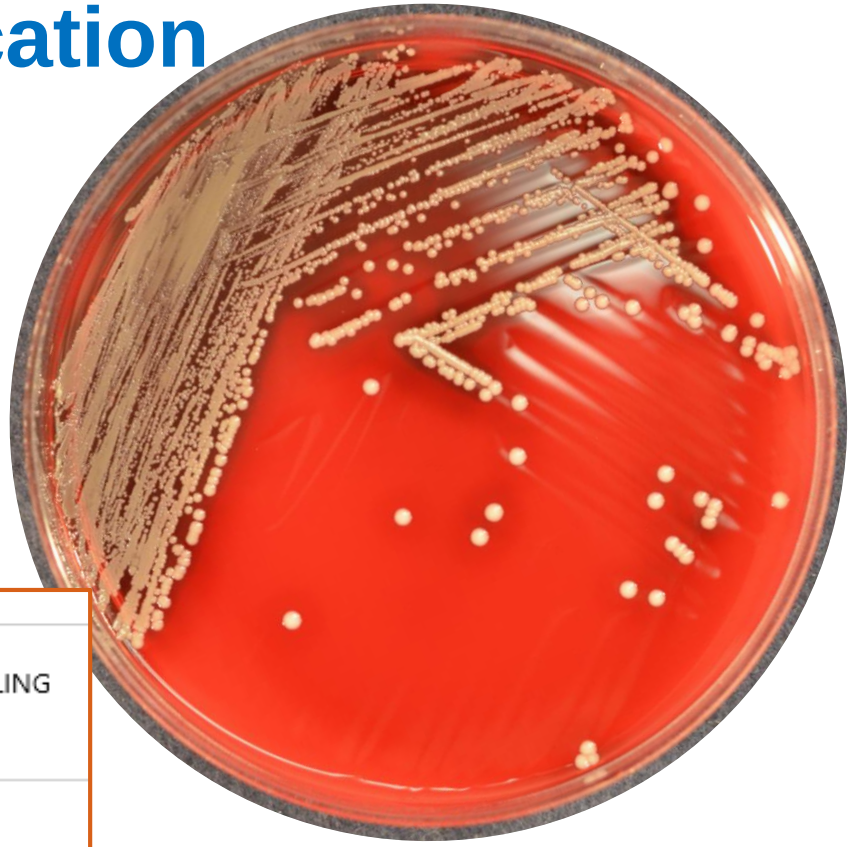
Date/Time	NAME	SPECIMEN	CULTURE
04/15/21 0700	BLOOD CULTURE	BLOOD ARM, LEFT	POSITIVE SMEAR: GRAM POSITIVE COCCI RESEMBLING STAPHYLOCOCCI growth in both bottles
04/15/21 0700	BLOOD CULTURE MOLECULAR DETECTION	BLOOD ARM, LEFT	Staphylococcus aureus detected mecA/mecC gene not detected Methicillin susceptible

Blood Culture [439351753]	Component	Value
(Abnormal)	Blood Culture	Staphylococcus epidermidis ! P
Blood Peripheral	Blood Culture	Gram Positive Rods ! P

Blood Culture [439351755]	Component	Value
Blood Peripheral	Blood Culture	Negative 2 Days P

BCGP NAAT [439495861]	Component	Value
Blood Peripheral	Staphylococcus species	Not Detected
	Staphylococcus aureus	Not Detected
	Staphylococcus epidermidis	Not Detected
	Staphylococcus lugdunensis	Not Detected
	Streptococcus species	Not Detected
	Streptococcus anginosus (Milleri) group	Not Detected
	Streptococcus agalactiae (Strep Group B)	Not Detected
	Streptococcus pyogenes (Strep Group A)	Not Detected
	Streptococcus pneumoniae	Not Detected
	Enterococcus faecalis	Not Detected
	Enterococcus faecium	Not Detected
	Listeria species	Not Detected
	mecA gene (Methicillin) resistance NAAT	Not Applicable
	Van-A gene (Vancomycin) resistance NAAT	Not Applicable
	Van-B gene (Vancomycin) resistance NAAT	Not Applicable

Implementing Genotypic Susceptibility Testing: Reporting and Communication



Reporting

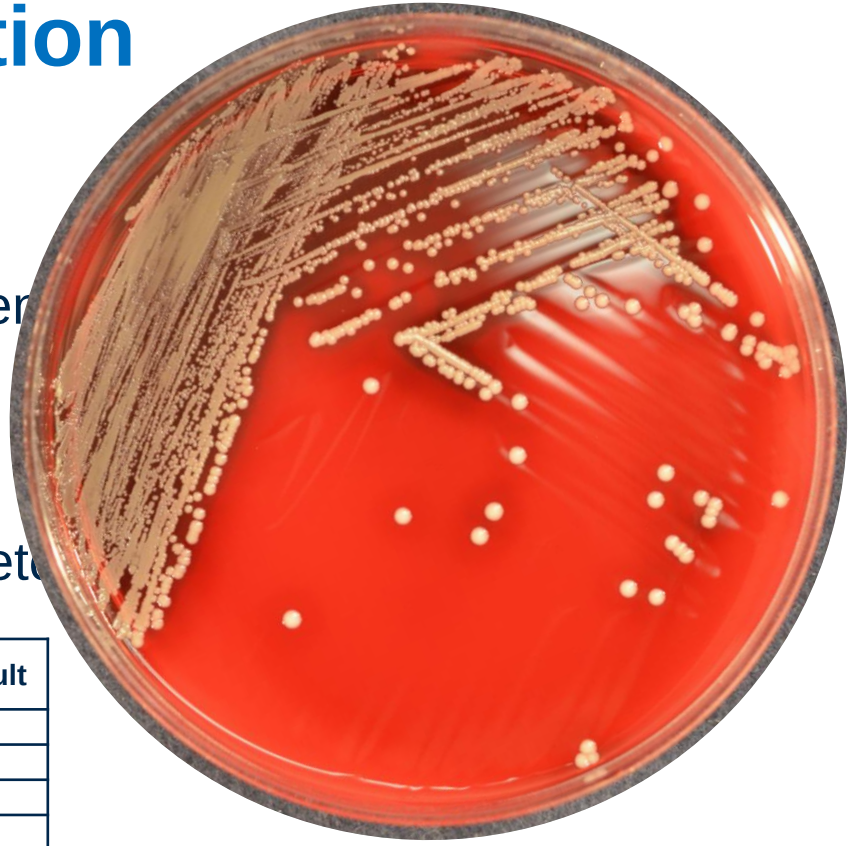
- Incorporate into culture results vs separate line list
  - Considerations:
    - LIS/EMR capabilities
    - Billing
- Incorporate interpretation comments into reports
  - Determine in collaboration with antimicrobial stewardship group
    - “methicillin susceptible/resistant”
    - “vancomycin susceptible/resistant”
    - “ESBL producer”
    - “resistant to carbapenem antibiotics”

CULTURE
POSITIVE SMEAR: GRAM POSITIVE COCCI RESEMBLING STAPHYLOCOCCI growth in both bottles
Staphylococcus aureus detected mecA/mecC gene not detected Methicillin susceptible

Initial go-live communication with physicians

- Emphasize preliminary nature of results
- Discuss possible discrepancies and expected outcomes

Implementing Genotypic Susceptibility Testing: Laboratory Implementation



Phenotypic AST remains the gold standard

Implement checks in SOPs to confirm genotypic / phenotypic agreemen
- Check **PRIOR** to reporting phenotypic AST
- Establish basic procedures for work up of discrepancies
- Communication pending resolution
  - Clinical team, section director, management (TS, lead, supe, etc

Relevant Organisms	Resistance Genes	BCID Gene Result	Antimicrobial	Expected AST Result
BCID-GP				
***Staphylococcus* species**	mecA mecC	Detected	Oxacillin and/or cefoxitin	Resistant
		Not Detected		Susceptible
***Enterococcus* species**	vanA vanB	Detected	Vancomycin	Resistant
		Not Detected		Susceptible
BCID-GN				
Enterobacterales	CTX-M	Detected	Ceftriaxone, cefotaxime	Resistant
Enterobacterales, *P. aeruginosa*, *Acinetobacter* species	KPC NDM VIM IMP OXA23/48	Detected	Meropenem AND Ertapenem	Resistant

Genotypic to Phenotypic Comparison Scenarios

1. Genotype correlates with phenotype
   No further testing required

2. AMR gene detected; isolate is phenotypically susceptible

3. AMR gene not detected; isolate is phenotypically resistant

→ Require additional follow up

Genotypic/Phenotypic Conflicts
Approaches to Troubleshooting

Documentation

- BCID module, panel lot
- Blood culture bottle type, lot
- Any additional necessary for vendor troubleshooting

Initial Work Up

- Confirm purity of culture and AST set up
- Confirm organism ID as appropriate
- Rule out clerical errors; double check BCID reporting
- Review patient history / AST for similar results

BCID AMR GENE CONFLICT TROUBLESHOOTING QUICK GUIDE

Initial steps performed by bench tech

- Confirm pure culture; if mixed, work up separately
- Confirm organism ID
- Rule out clerical errors
- Review patient history / past AST

If conflict is not resolved

- Document module and lot number of BCID panel used
- Document lot number of blood culture bottle
- **Notify management and section director**
- Hold AST
- Perform below steps in consultation with section director / mgmt

Genotypic to Phenotypic Discrepancy Cases

What to do when the results don’t agree

Genotypic/Phenotypic Discrepancies: Case 1

Gram:

Gram negative rods

BCID:

Proteus mirabilis detected
KPC, NDM, IMP, VIM, OXA23/48 not detected

Culture:

Proteus mirabilis

AST:

Amikacin	<=8	S
Amp/Sulb	>16/8	R
Ceftriaxone	>=4	R
Ertapenem	<=0.25	S
Imipenem	>2	R
Gentamicin	>8	R
Levofloxacin	>4	R
Pip/Tazo	32/4	I
Trim/Sulfa	>2/38	R

Discrepancy

Carbapenemase gene not detected
Ertapenem S / Imipenem R

Reason

Proteus, Morganella, Providencia have intrinsically elevated IMI MIC
CLSI M100, Table 2A-1, Comment 25

Troubleshooting Considerations

None

Resolution

None
Does not need management review

(Biological) Sources of Genotypic/Phenotypic Conflicts

Reason 1: Alternative mechanism for resistance

Troubleshooting:

- Rule out alternative explanations
- Understand resistance mechanisms outside of detected AMR genes (M100 very helpful!)

Organism	Phenotypic AST	Genotypic AST	Mechanism
E. cloacae complex	Ertapenem R	No carbapenemase gene detected	Derepressed ampC + porin mutation
Acinetobacter baumannii	Meropenem R	No carbapenemase gene detected	OXA-23 or OXA-24/40 not detected by panel
Staphylococcus aureus	Oxacillin R	*mecA* negative	*mecC* Staph β-lactamase hyperproduction (BORSA)

Genotypic/Phenotypic Discrepancies: Case 2

Gram:

Gram positive cocci

BCID:

Staphylococcus epidermidis detected
mecA/C gene detected

Culture:

Staphylococcus epidermidis

AST:

Clindamycin	<=0.5	R
Daptomycin	<=1	S
Erythromycin	>4	R
Gentamicin	<=2	S
Linezolid	2	S
Oxacillin	1	S
Rifampin	<=0.5	S
Tetracycline	<=0.5	S
Trim/Sulfa	<1/19	S
Vancomycin	1	S

Discrepancy

mecA/C gene detected
Methicillin susceptible

Reason

Incorrect breakpoints used

Troubleshooting Considerations

Confirm correct breakpoints with CLSI M100 document

Resolution

Report using correct breakpoints

***Staphylococcus* species**	**Oxacillin Interpretive Categories and MIC Breakpoints**		
	S	I	R
S. aureus and *S. lugdunensis*	≤2	-	≥4
S. epidermidis	≤0.5	-	≥1
S. pseudintermedius, S. coagulans, and *S. schleiferi*	≤0.5	-	≥1

Genotypic/Phenotypic Discrepancies: Case 3

Gram:

Gram positive cocci

BCID:

Staphylococcus aureus detected
Staphylococcus epidermidis detected
probable contaminant
mecA/C gene detected

Culture:

Staphylococcus aureus
Staphylococcus epidermidis, probable contaminant

AST:

Staphylococcus aureus

Clindamycin	<=0.5	R
Daptomycin	<=1	S
Erythromycin	>4	R
Gentamicin	<=2	S
Linezolid	2	S
Oxacillin	1	S
Rifampin	<=0.5	S
Trim/Sulfa	<1/19	S
Vancomycin	1	S

Discrepancy
mecA/C gene detected
Methicillin susceptible

Reason
mecA/C carried by *S. epidermidis*

Troubleshooting Considerations
Confirm methicillin R in CoNS before reporting

Resolution
Multiple staph detection reported with comment
If CoNS is methicillin R, no conflict

BLOOD ARM, LEFT	Staphylococcus aureus detected Staphylococcus epidermidis detected mecA/mecC gene detected When multiple staphylococcal species are present, association of the mecA/C resistance gene with a specific organism cannot be determined.

(Biological) Sources of Genotypic/Phenotypic Conflicts

Reason 1: Alternative reason for resistance

- Troubleshooting:
  - Rule out alternative explanations
  - Understand resistance mechanisms outside of detected AMR genes (M100 very helpful!)

Reason 2: AMR gene / reported organism mismatch

- Troubleshooting: ID and AST on all organisms in culture
  - Usually straightforward in BCx, can be complicated in other sources

Genotypic/Phenotypic Discrepancies: Case 4

Gram:

Gram positive cocci

BCID:

Staphylococcus aureus detected
mecA/C gene detected

Culture:

Staphylococcus aureus

AST:

Clindamycin	<=0.5	R
Daptomycin	<=1	S
Erythromycin	>4	R
Gentamicin	<=2	S
Linezolid	2	S
Oxacillin	1	S
Rifampin	<=0.5	S
Tetracycline	<=0.5	S
Trim/Sulfa	<1/19	S
Vancomycin	1	S

Discrepancy

mecA/C gene detected
Methicillin susceptible

Reason

Mixed culture with coagulase negative *Staphylococcus* species (CoNS)
Hetero-resistant population
Gene truncation / mutation

Troubleshooting Considerations

Repeat AST with alternative method (eg, cefoxitin disk) as available
Consider testing bottle by alternative MRSA test
Heavy subculture to find CoNS
Subculture to BAP with FOX disk
Perform PBP2a antigen test

Resolution

Colonies found within the FOX disk zone, IDed as *S. aureus*
Report MRSA

(Biological) Sources of Genotypic/Phenotypic Conflicts

Reason 1: Alternative reason for resistance

Troubleshooting:

- Rule out alternative explanations
- Understand resistance mechanisms outside of detected AMR genes (M100 very helpful!)

Reason 2: AMR gene / reported organism mismatch

Troubleshooting: ID and AST on all organisms in culture

- Usually straightforward in BCx, can be complicated in other sources

Reason 3: Hetero-resistance

Troubleshooting: heavy subculture to BAP with disk (or screen plate) to identify subpopulation

Genotypic/Phenotypic Discrepancies: Case 5

Gram:

Gram positive cocci

BCID:

Enterococcus faecium detected
vanA/B gene detected

Culture:

Enterococcus faecium

AST:

Ampicillin	>8	R
Daptomycin	4	S
Gent Synergy	<=500	S
Linezolid		S
Rifampin		R
Tetracycline	>8	R
Vancomycin	<=0.5	S

Discrepancy

vanA/B gene detected
Vancomycin susceptible

Reason

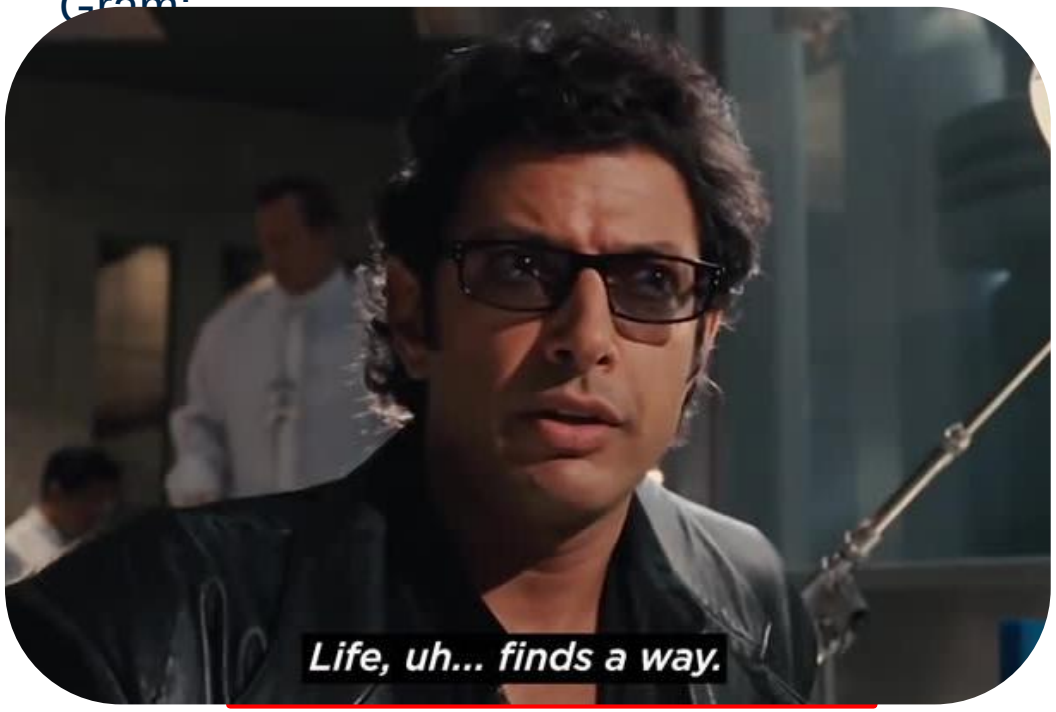
Hetero-resistant population
Gene deletion/mutation

Troubleshooting Considerations

Consider mixed population with multiple *Enterococcus* species
Confirm species identification / culture purity
Repeat vancomycin AST by an alternative method (eg, strip, vanc screening plate)
Consider detection of *vanA/B* gene by alternative method, if available

Genotypic/Phenotypic Discrepancies: Case 5

Gram:



Troubleshooting Results

Repeat AST (automated system): same results

Subculture to vanc screening plates (6ug/mL): no growth

Alternative AST method performed: vanc S

vanA gene detected by alternative molecular method

Patient treated with vancomycin

→ clinical failure

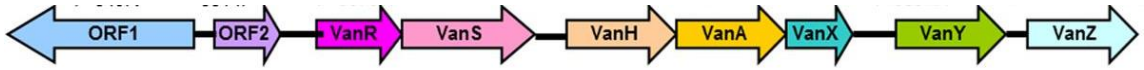
Organism was re-isolated from the patient following failure

vanA detected, vancomycin resistant

Vancomycin Variable Enterococci (VVE)

vanA gene cluster

large deletion in *vanRS* promotor



secondary DNA structure change led to constitutively expressed *vanA* gene

Report initial isolate as vancomycin R

(Biological) Sources of Genotypic/Phenotypic Conflicts

Reason 1: Alternative reason for resistance

Troubleshooting:

- Rule out alternative explanations
- Understand resistance mechanisms outside of detected AMR genes (M100 very helpful!)

Reason 2: AMR gene / reported organism mismatch

Troubleshooting: ID and AST on all organisms in culture

- Usually straightforward in BCx, can be complicated in other sources

Reason 3: Hetero-resistance

Troubleshooting: heavy subculture to BAP with disk (or screen plate) to identify subpopulation

Reason 4: Mutations in AMR gene, plasmid kicked out, reversion of resistance, other wacky t

Troubleshooting:

- Rule out alternative explanations
- Literature review of reported cases

Troubleshooting Genotypic/Phenotypic Conflicts

You don’t have to figure out the reason for discrepancy

Investigate the basic stuff

Check for clerical/breakpoint errors
Repeat phenotypic AST, via alternative method if available (+ genotypic if warranted)
Perform available phenotypic method detection methods (PBP2a, mCIM, CarbaNP)
Subculture for heteroresistant population

Troubleshooting Genotypic/Phenotypic Conflicts

You don’t have to figure out the reason for discrepanc

Establish a reporting scheme for when discrepancies are not resolved

Organism	Phenotypic AST	Genotypic AST	Reporting
Staphylococcus spp	Oxacillin / cefoxitin S	*mecA/C* detected	Isolates that test positive for *mecA* or PBP2a or resistant by any of the recommended phenotypic methods should be reported as methicillin (oxacillin) resistant

Troubleshooting Genotypic/Phenotypic Conflicts

You don’t have to figure out the reason for discrepanc

Establish a reporting scheme for when discrepancies are not resolved

Organism	Phenotypic AST	Genotypic AST	Reporting
Staphylococcus spp	Oxacillin / cefoxitin S	*mecA/C* detected	Isolates that test positive for *mecA* or PBP2a or resistant by any of the recommended phenotypic methods should be reported as methicillin (oxacillin) resistant
Enterococcus spp	Vancomycin S	*vanA/B* detected	Vancomycin R

Troubleshooting Genotypic/Phenotypic Conflicts

You don't have to figure out the reason for discrepanc

Establish a reporting scheme for when discrepancies are not resolved

Organism	Phenotypic AST	Genotypic AST	Reporting
Staphylococcus spp	Oxacillin / cefoxitin S	*mecA/C* detected	Isolates that test positive for *mecA* or PBP2a or resistant by any of the recommended phenotypic methods should be reported as methicillin (oxacillin) resistant
Enterococcus spp	Vancomycin S	*vanA/B* detected	Vancomycin R
Enterobacterales	Meropenem S	KPC, NDM, VIM, IMP, OXA detected	CLSI: Send to reference lab for AST via reference BMD Report AST as tested + AMR gene + caution comment

Troubleshooting Genotypic/Phenotypic Conflicts

CLSI M100 ed35 (2025) Appendix G Table G3

If the discrepancy is not resolved, **repeat AST should be performed using a reference method** and the **conflicting genotypic and phenotypic testing results should both be reported** along with a comment advising caution;

current clinical and laboratory evidence is insufficient to conclude whether carbapenem monotherapy of carbapenemase-carrying strains with an MIC in the S range **will be effective**, or whether the molecular assays are completely accurate.

Orga		
Staph *s* spp		
Entero spp		
Enter es	VIM,IMP ,OXA detected	Report AST as tested + gene + caution comment WDL: Report all cephems and carbapenems as R

Troubleshooting Genotypic/Phenotypic Conflicts

You don't have to figure out the reason for discrepanc

Establish a reporting scheme for when discrepancies are not resolved

Organism	Phenotypic AST	Genotypic AST	Reporting
Staphylococcus spp	Oxacillin / cefoxitin S	*mecA/C* detected	Isolates that test positive for *mecA* or PBP2a or resistant by any of the recommended phenotypic methods should be reported as methicillin (oxacillin) resistant
Enterococcus spp	Vancomycin S	*vanA/B* detected	Vancomycin R
Enterobacterales	Meropenem S	KPC, NDM, VIM, IMP, OXA detected	CLSI: Send to reference lab for AST via refBMD Report AST as tested + geno + caution comment WDL: Report all cephems and carbapenems as R

Troubleshooting Discordant Genotypic and Phenotypic Results

Resources:

CLSI M100 Appendix G: Using Molecular Assays for Resistance Detection

Yee R, et al. *J Clin Micro* 2021 (PMID 33441396)

Table G2. Strategies for Reporting Vancomycin Results When Using Molecular and Phenotypic AST Methods for *Enterococcus* spp.

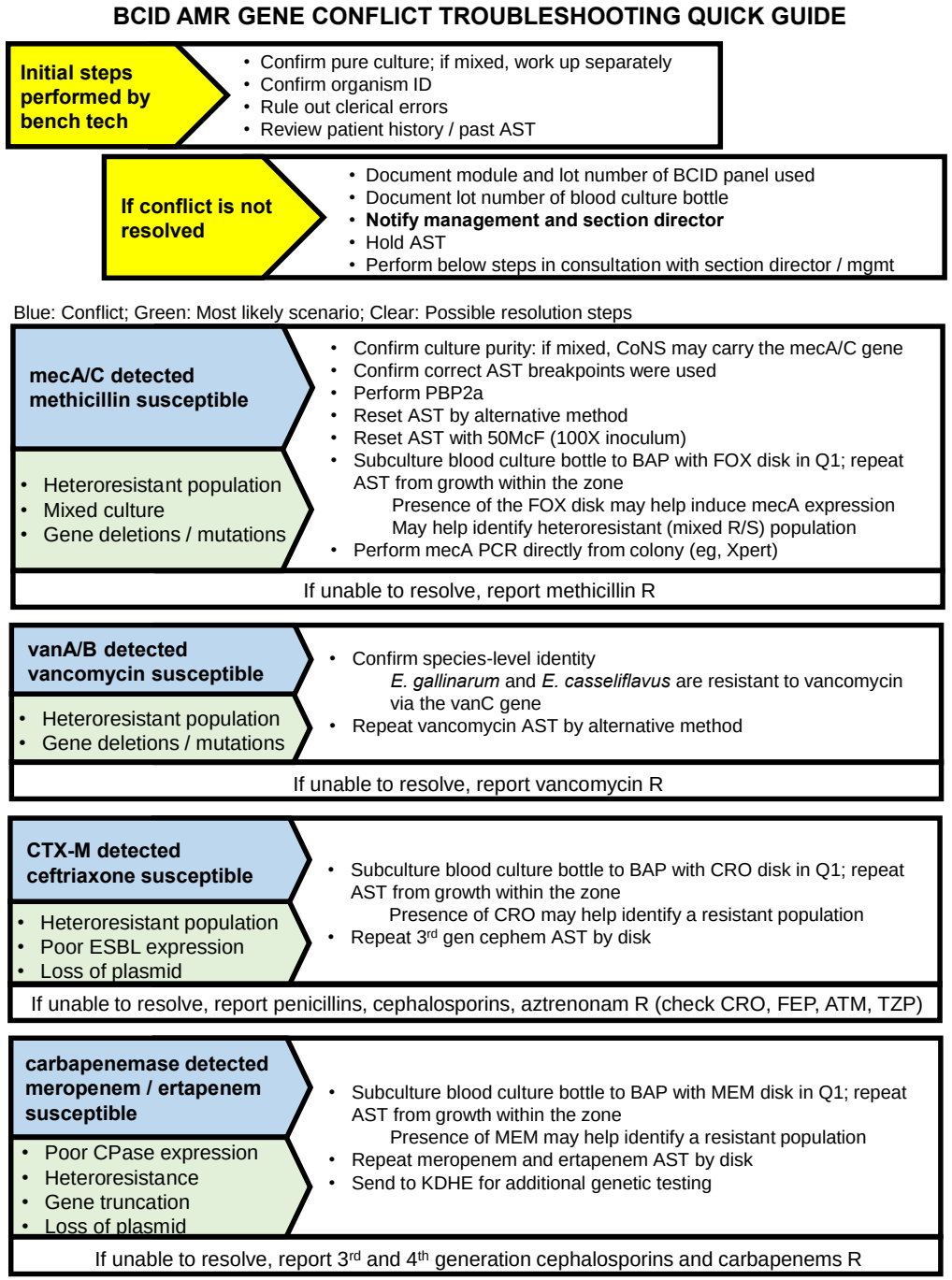
Indication	Resistance Mechanism(s)	Methods	Specimen Types	Results		Suggestions for Resolution	Report as:	Comments[a]
				Resistance Mechanism(s) Detected	Phenotypic AST (if tested)			
Detection of VRE	*vanA* *vanB*	NAAT or array hybridization technology	Blood culture broth or surveillance cultures	*vanA* and/or *vanB* detected	Vancomycin R	N/A	Report phenotypic result as found (if available); consider reporting presence of molecular target per institutional protocol.	1–3
				vanA and/or *vanB* not detected	Vancomycin S	N/A	Report phenotypic result as found (if available); consider reporting presence of molecular target per institutional protocol.	
				vanA and/or *vanB* detected	Vancomycin S	Confirm isolate identification to species level (eg, *E. faecalis*) and repeat AST. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as vancomycin R.	1–3
				vanA and/or *vanB* not detected	Vancomycin R	Confirm isolate identification to species level (eg, *E. faecalis*) and repeat AST. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as vancomycin R.	4

Troubleshooting Discordant Genotypic and Phenotypic Results

Resources:

CLSI M100 Appendix G: Using Molecular Assays for Resistance Detection

Yee R, et al. *J Clin Micro* 2021 (PMID 33441396)



Interpreting Discordant Genotypic and Phenotypic Results

It's complicated!

Goal of susceptibility testing is to predict treatment success/failure for the patient

Detection of a resistance marker does not necessarily predict therapeutic failure of an antibiotic

- Nonfunctional gene due to mutation or truncation
- Expression at clinically insignificant levels

Absence of a genetic marker does not necessarily indicate susceptibility

- Resistance due to alternative mechanisms not detected by method
- Technical issues with detection (target below limit of detection, amplification inhibition)

Increased sensitivity of molecular methods over traditional culture/AST may contribute to discre

- Low gene expression in culture
- Mixed populations / heteroresistance
- Poor organism growth, leading to erroneously low MICs

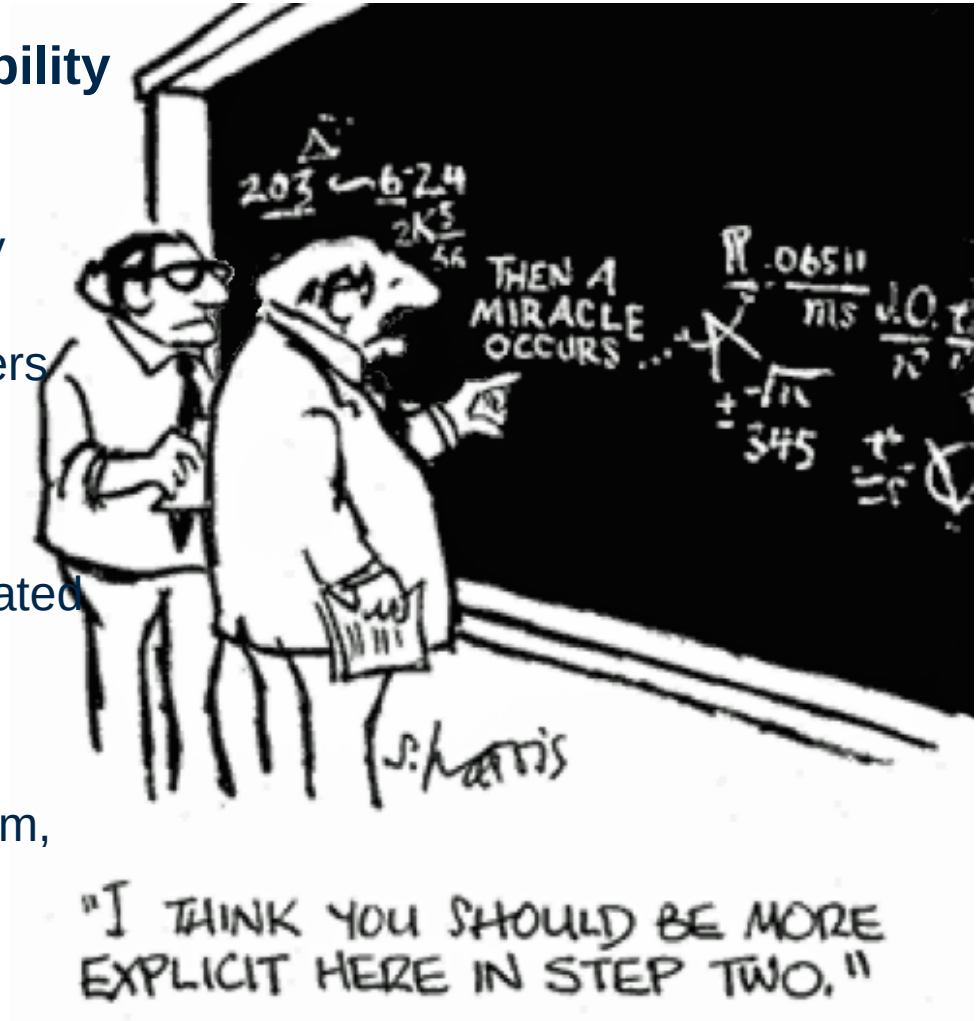
Interpreting Discordant Genotypic and Phenotypic Results

Keys to successful implementation of genotypic susceptibility testing

Established defined workflows for troubleshooting commonly identified discrepancies
- Guidelines for bench technologists and microbiology leaders
- Timely reactions to identified discrepancies

Balance need for accuracy and investigation with the associated increased cost and TAT

Communicate with physicians, antimicrobial stewardship team, pharmacy
- Transparency on expected discrepancies, troubleshooting plans
- Active communication on a case-specific basis



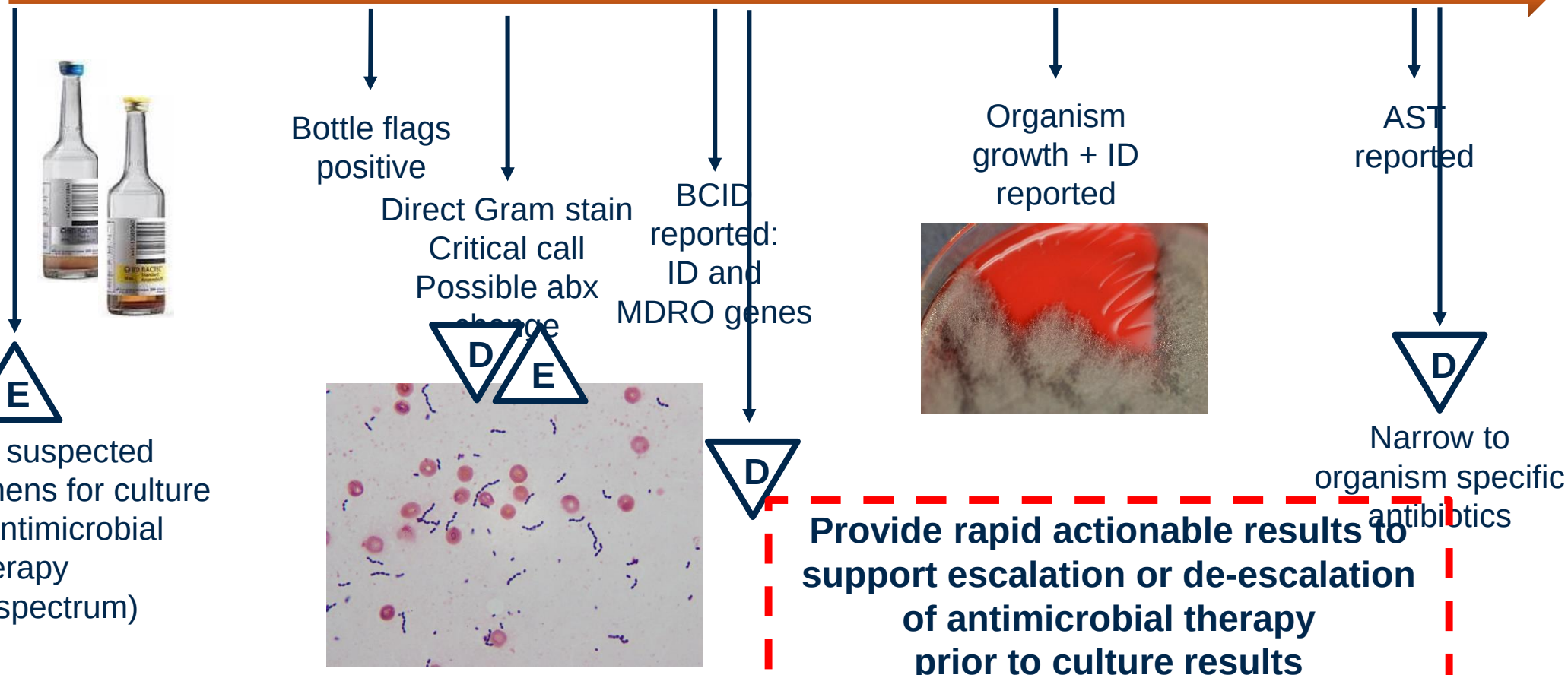
Questions?

MEDICAL COLLEGE OF WISCONSIN

Clinical Utility

Standard Blood Culture Workflow

Blood culture incubation: 5d
Most pathogens pos: 1-2d



Provide rapid actionable results to support escalation or de-escalation of antimicrobial therapy prior to culture results

Genotypic/Phenotypic Discrepancies: Case 1

Gram:

Gram negative rods

BCID:

Enterobacter cloacae detected
KPC, NDM, IMP, VIM, OXA23/48 not detected

Culture:

Enterobacter cloacae

AST:

Amikacin	<=8	S
Amp/Sulb	>16/8	R
Ceftriaxone	>=4	R
Ertapenem	>2	R
Gentamicin	>8	R
Levofloxacin	>4	R
Pip/Tazo	32/4	I
Trim/Sulfa	>2/38	R

Discrepancy

Carbapenemase gene not detected
Ertapenem R

Reason

Alternative mechanism

AmpC + Porin
other carbapenemase gene not on panel

Troubleshooting Considerations

Consider phenotypic test for carbapenemase activity (eg, mCIM)

Resolution

None
Does not need management review
Send to state public health lab for further testing, as required

(Biological) Reasons for Genotypic/Phenotypic Conflicts

General Reason 1: AMR gene / reported organism mismatch

Solution: ID and AST on all organisms in culture

General Reason 2: Alternative reason for resistance

Solution: understand resistance mechanisms outside of detected AMR genes

General Reason 3: Heteroresistance

Solution: identify heteroresistant population by subculture w/ abx

General Reason 4: Mutations in AMR gene

Solution: depends…

Genotypic/Phenotypic Discrepancies: Case 5

Gram:

Gram negative rods

BCID:

Klebsiella pneumoniae detected
KPC detected

Culture:

Klebsiella pneumoniae

AST:

Amikacin	<=8	S
Amp/Sulb	>16/8	R
Ceftriaxone	>=4	R
Ertapenem	<=0.25	S
Gentamicin	>8	R
Levofloxacin	>4	R
Pip/Tazo	32/4	I
Trim/Sulfa	>2/38	R

Discrepancy

Carbapenemase gene detected / ertapenem

Reason

Heteroresistant population
Poor KPC expression
Gene truncation
Loss of plasmid upon subculture

Troubleshooting Considerations

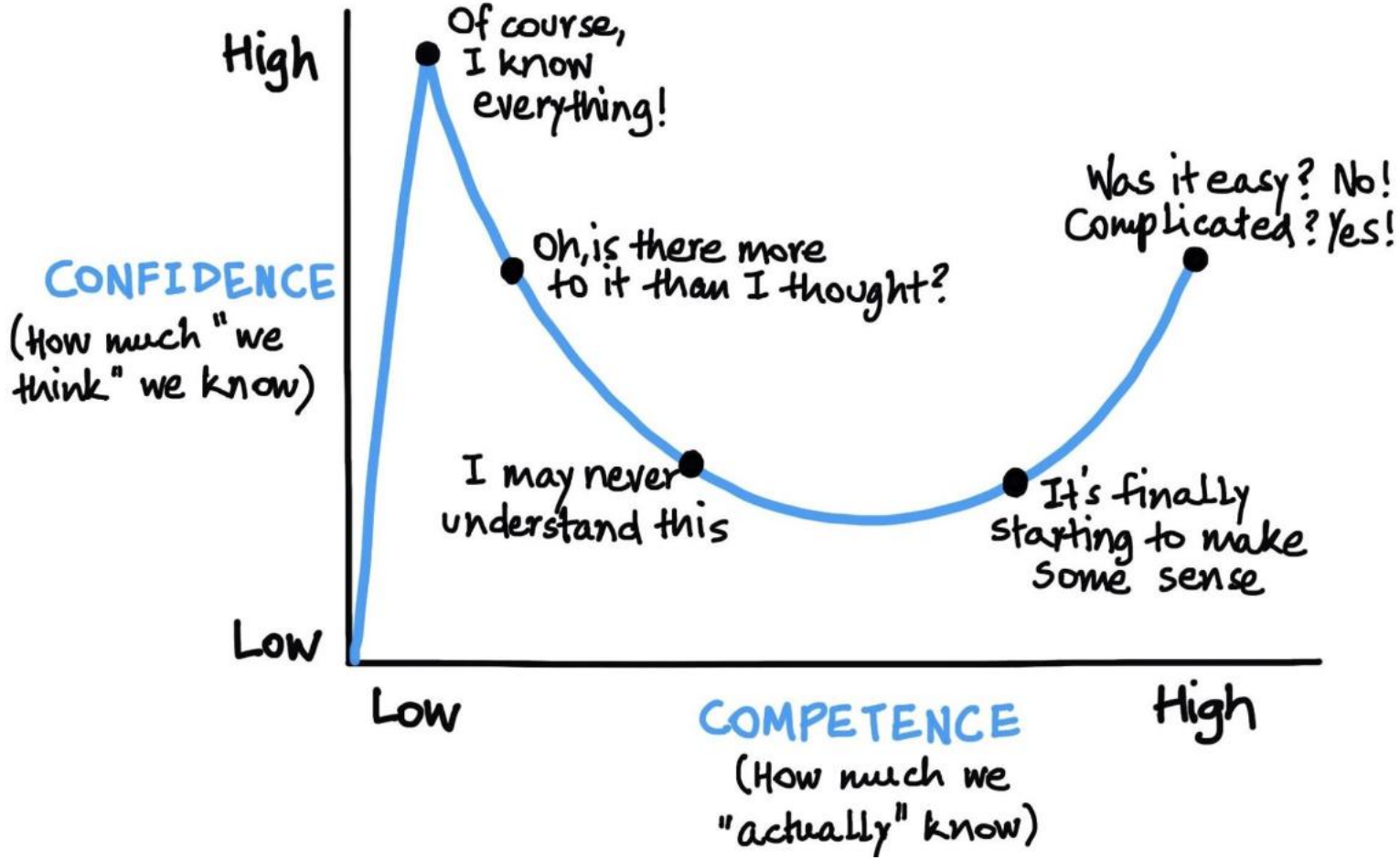
Repeat AST by disk/strip to confirm (ertapenem & meropenem)
Repeat BCID to confirm (perform alternative NAAT, if available)
Perform phenotypic test for CPase activity (eg, mCIM, CarbaNP)
Subculture bottle in presence of ERT and/or MEM disk
    Look for organisms within zone

Resolution

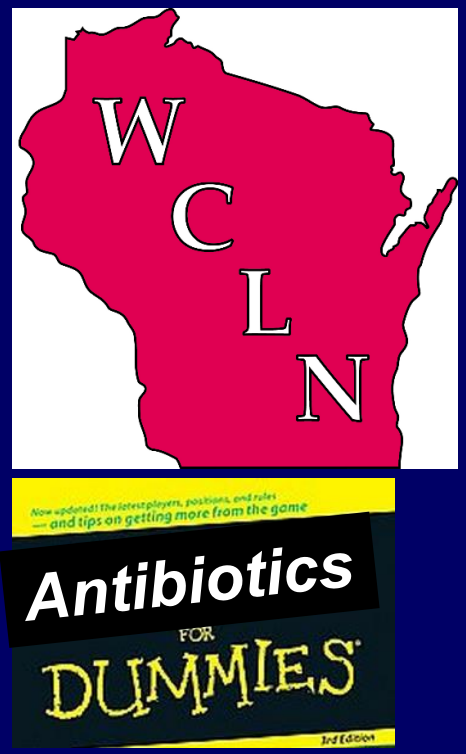
Option 1 (WDL): Report all cephalosporins and carbapenems as R
Option 2 (CLSI): Send to reference lab for AST via refBMD
Option 3 (CLSI): Report AST as tested + geno + caution comment

The Dunning Kruger Effect

[vishal@safalniveshak.com]



Antibiotics 151 for Laboratory Laboratory Professionals, with Focus on Antimicrobial Resistance



Erik Munson
Marquette University
Wisconsin Clinical Laboratory Network
Laboratory Technical Advisory Group

…including myself

The presenter states no conflict of interest and has no financial relationship to disclose relevant to the content of this presentation.

OUTLINE

I. Factors to consider

II. General mechanisms of resistance

III. Resistance mechanisms vs. β-lactam agents

IV. Resistance mechanisms vs. non-β-lactam agents

<u>Major Focus Organisms</u>

Enterobacterales
Pseudomonas aeruginosa
Staphylococcus aureus
Streptococcus pneumoniae

"D#*%it, Jim,
I'm not a physician."

Introductory Comments

FACTORS TO CONSIDER

- Antimicrobial susceptibility testing (AST)
- Spectrum of therapy (empiric therapy)
- Availability

Cannot Enter Urinary Tract

macrolides
clindamycin
chloramphenicol

FACTORS TO CONSIDER

- Antimicrobial susceptibility testing (AST)
- Spectrum of therapy (empiric therapy)
- Availability

Cannot Enter Urinary Tract

macrolides
clindamycin
chloramphenicol

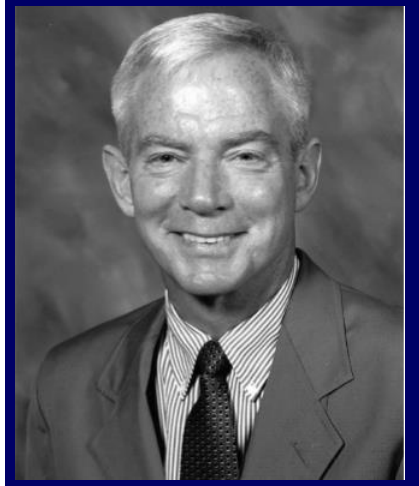
Cannot Enter CNS

fluoroquinolones
1st & 2nd generation cephems
clindamycin
macrolides
tetracycline

FACTORS TO CONSIDER

- Antimicrobial susceptibility testing (AST)
- Spectrum of therapy (empiric therapy)
- Availability
- Route of administration

Administration		Example
Medical Lingo	*Colloquial*	
IM	butt	ceftriaxone (also IV)
PO	oral	cephalexin
PO or parenteral	oral or IV	levofloxacin
parenteral	IV	vancomycin



FACTORS TO CONSIDER

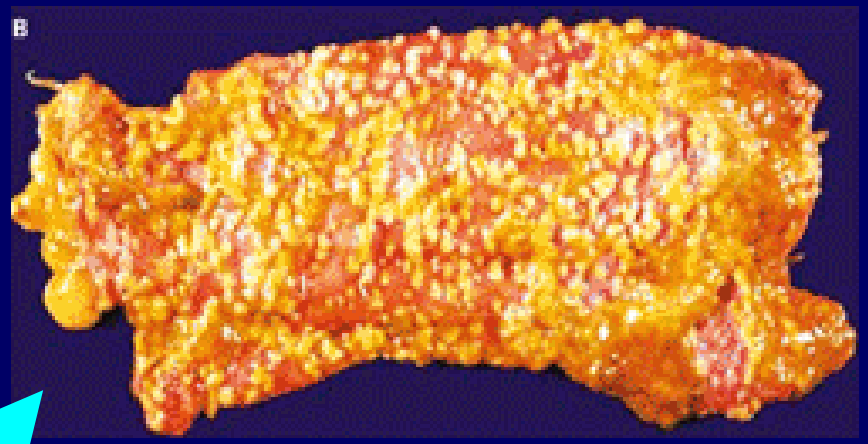
- Antimicrobial susceptibility testing (AST)
- Spectrum of therapy (empiric therapy)
- Availability
- Route of administration

Administration		**Example**
Medical Lingo	*Colloquial*	
IM	butt	ceftriaxone (also IV)
PO	oral	cephalexin
PO or parenteral	oral or IV	levofloxacin
parenteral	IV	vancomycin PO

FACTORS TO CONSIDER

- Antimicrobial susceptibility testing (AST)
- Spectrum of therapy (empiric therapy)
- Availability
- Route of administration

Administration		Example
Medical Lingo	*Colloquial*	
IM	butt	ceftriaxone (also IV)
PO	oral	cephalexin
PO or parenteral	oral or IV	levofloxacin
parenteral	IV	vancomycin PO



Pseudomembranous colitis caused by *Clostridioides difficile*[9]

FACTORS TO CONSIDER

- Antimicrobial susceptibility testing (AST)
- Spectrum of therapy (empiric therapy)
- Availability
- Route of administration
- Majority of excretion

Fluoroquinolone	**Percentage Excretion**	
	Renal	Biliary
levofloxacin	+++	-
ciprofloxacin	+++	+++++

FACTORS TO CONSIDER

- Antimicrobial susceptibility testing (AST)
- Spectrum of therapy (empiric therapy)
- Availability
- Route of administration
- Majority of excretion

Fluoroquinolone	Percentage Excretion	
	Renal	Biliary
levofloxacin	+++	-
ciprofloxacin	+++	+++++

Shigella spp. report

ampicillin

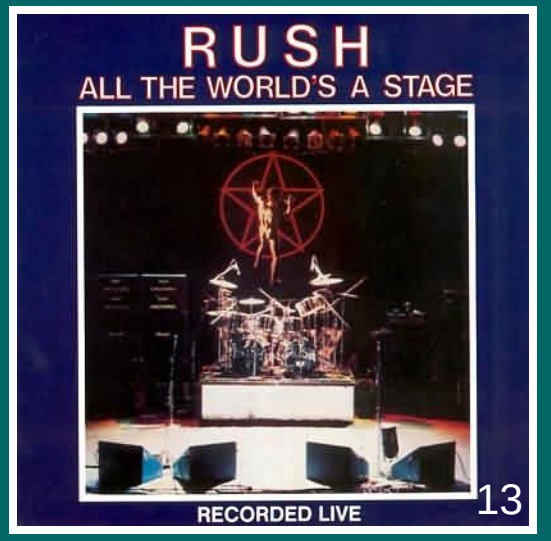
trimethoprim-sulfa

ciprofloxacin

FACTORS TO CONSIDER

- Antimicrobial susceptibility testing (AST)
- Spectrum of therapy (empiric therapy)
- Availability
- Route of administration
- Majority of excretion
- Dosing/half-life
- Synergy
- Side effects
- Kinetics
- Co$t
- Polymicrobial infections
- Cidal vs. static

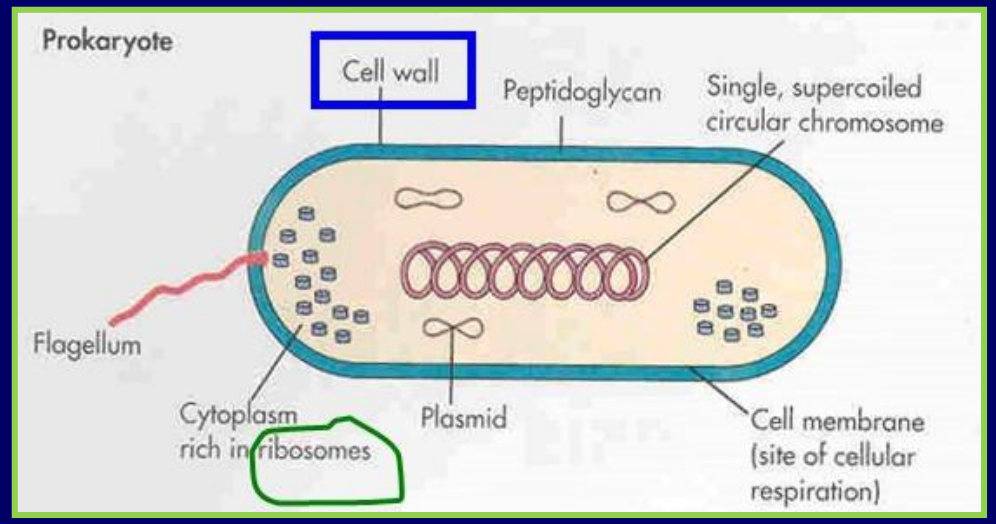
Setting the Stage

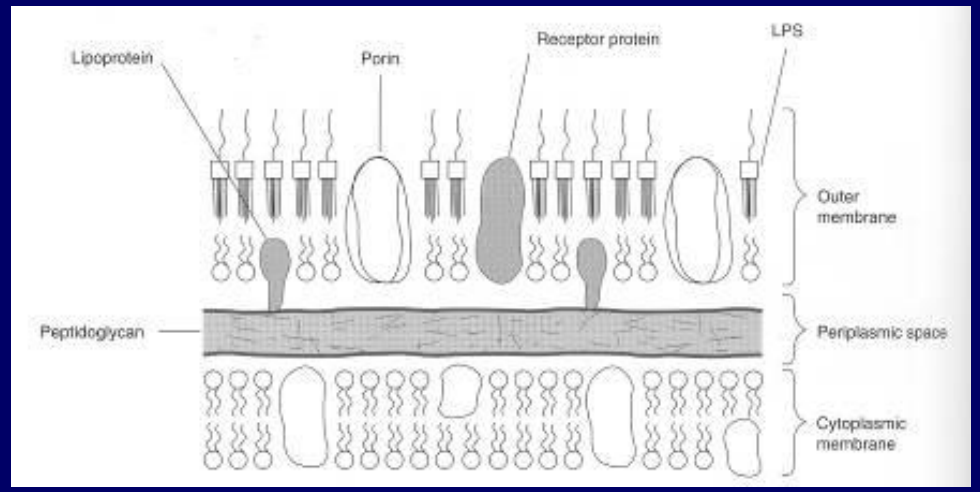


GENERAL MECHANISMS

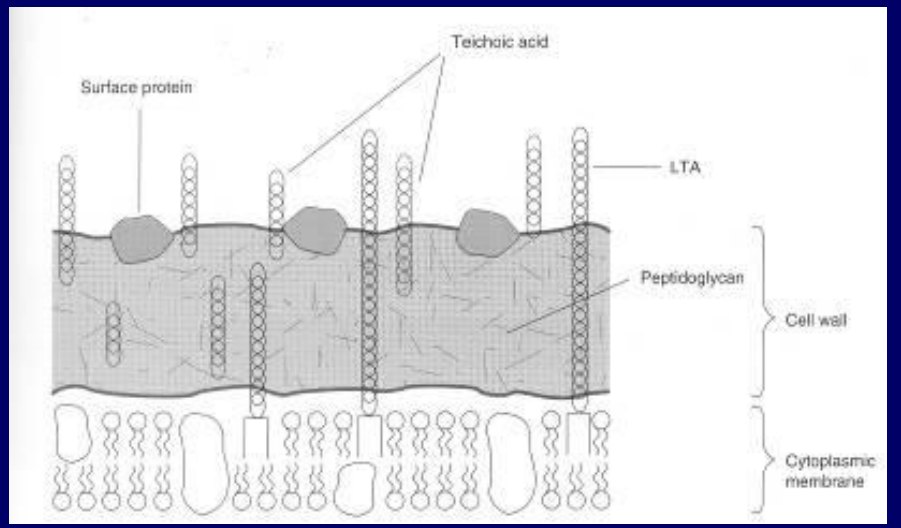
- Altered target
- Enzymatic inactivation
- Diminished penetration
- Efflux
- Altered physiology

IMPORTANT STRUCTURES



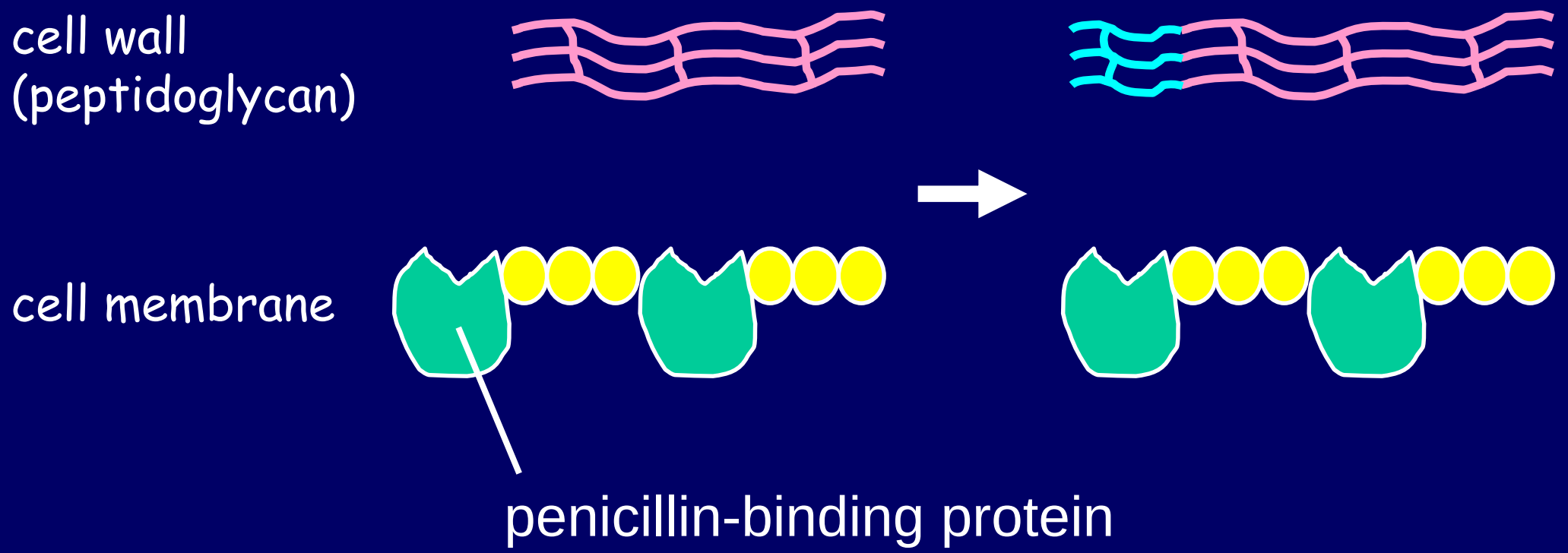


Gram negative



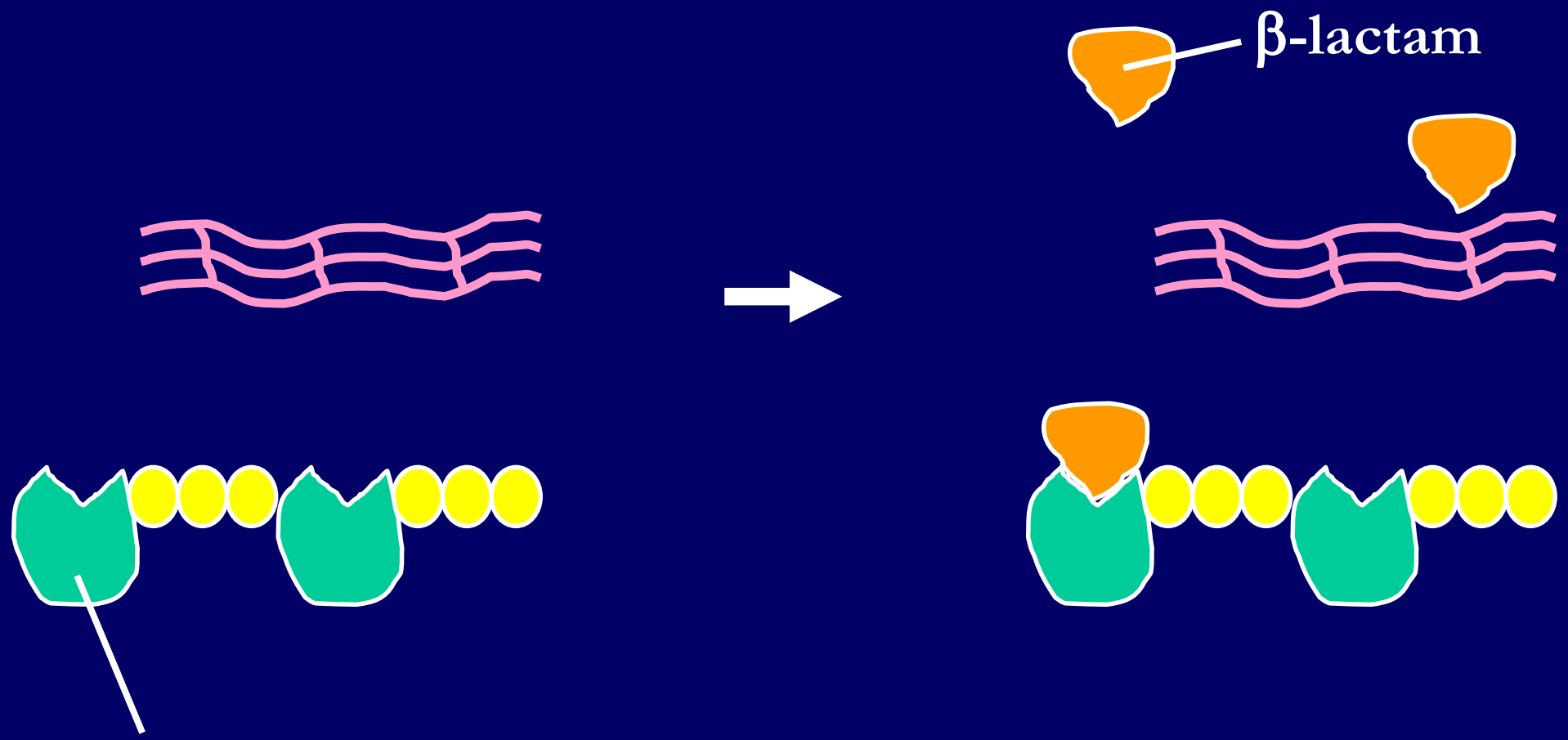
Gram positive

IMPORTANT STRUCTURES



Resistance in β-lactams

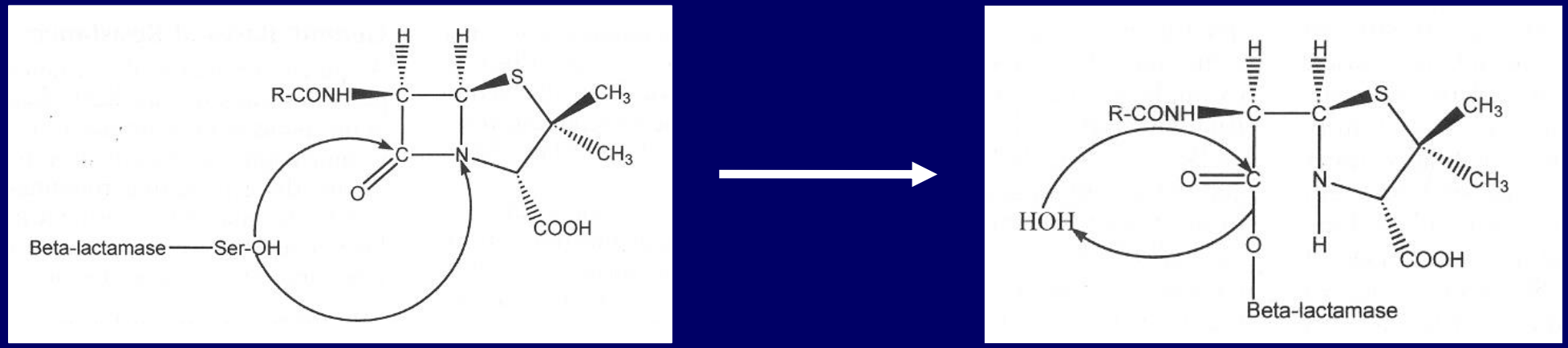
OUR FIRST TOPIC OF DISCUSSION



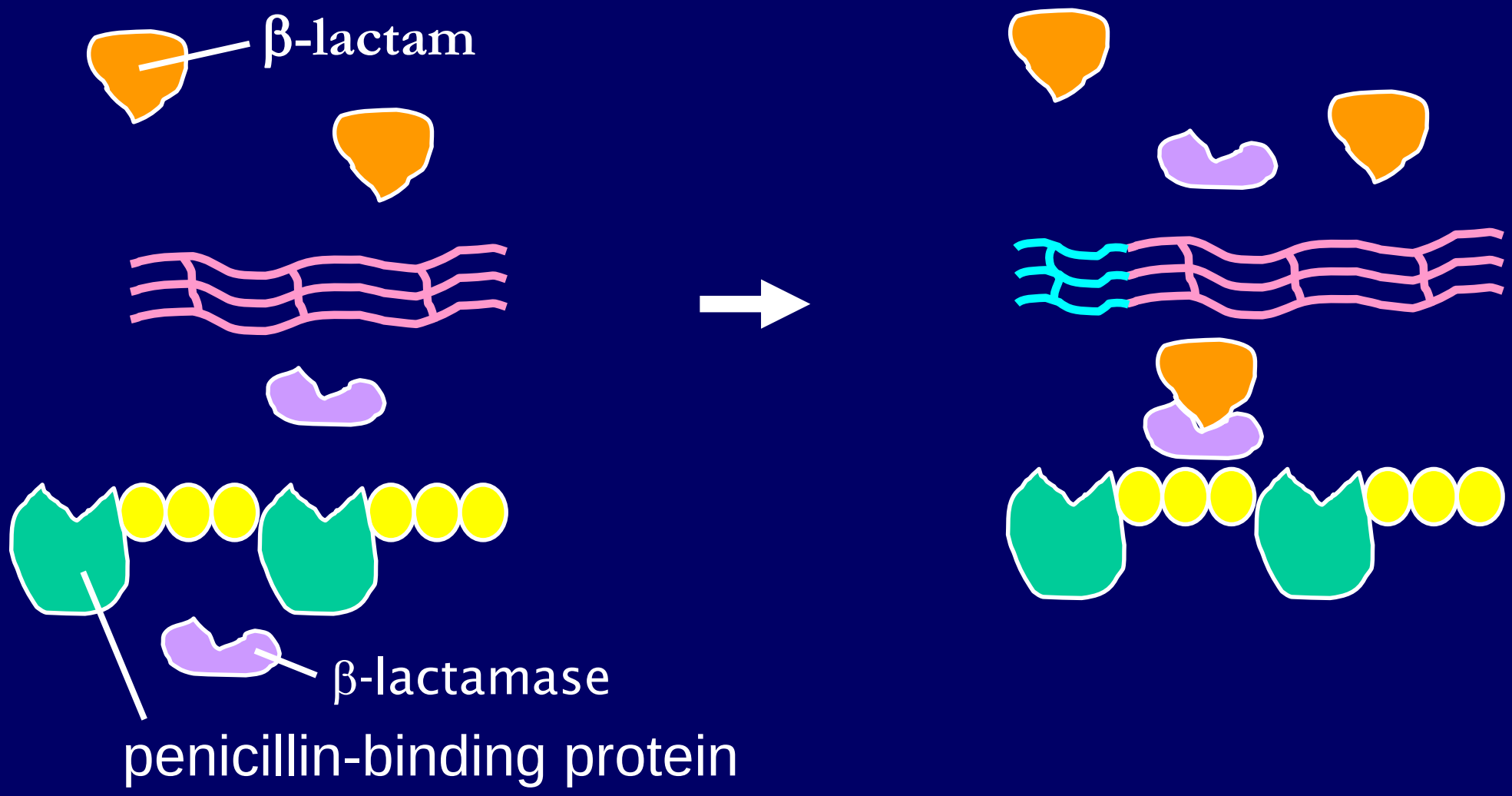
β-LACTAM RESISTANCE

- Mediated by β-lactamases

  >1000 individual enzymes have been reported



β-LACTAMASE CARTOON



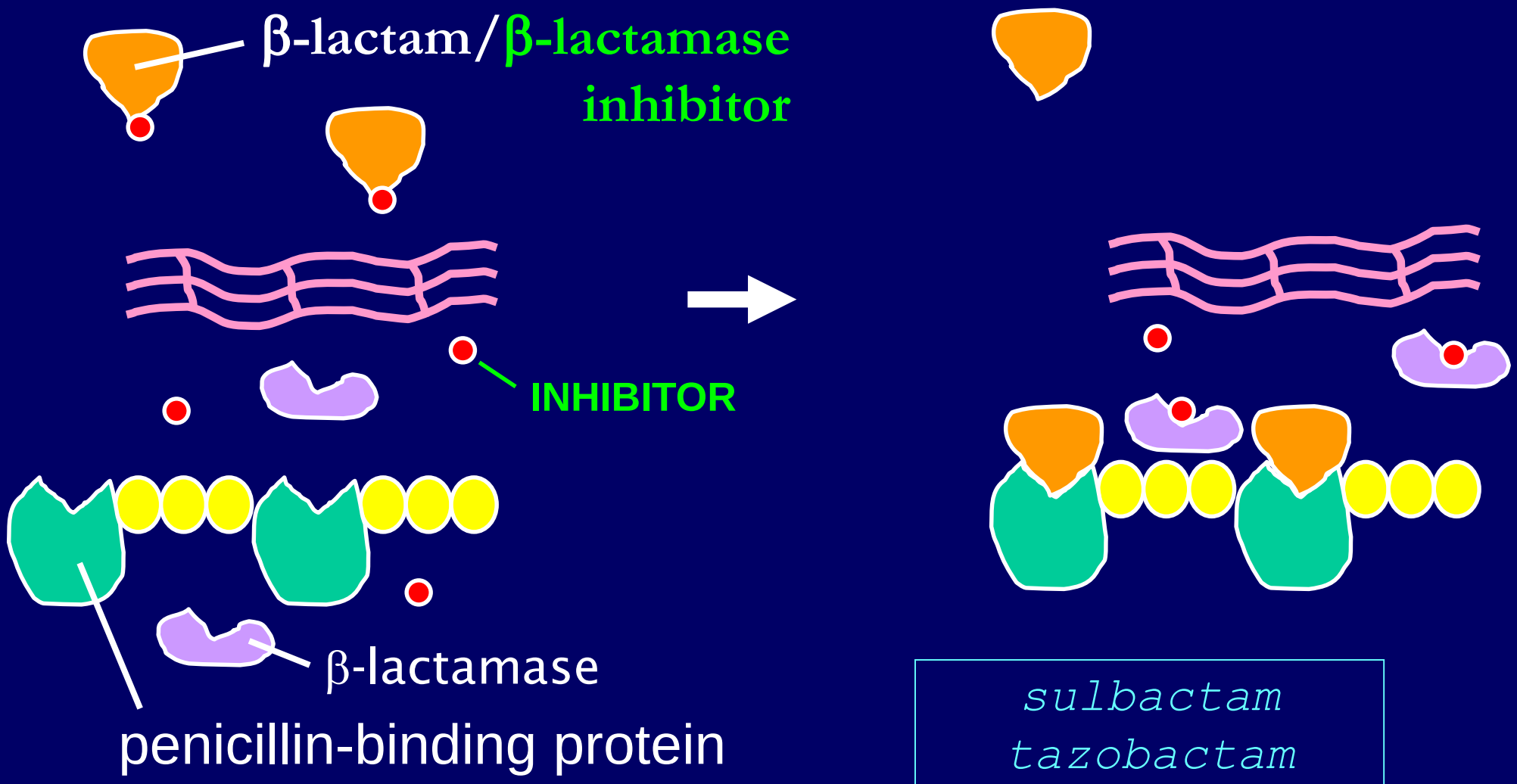
PENICILLIN CLASS

Subclass (if appropriate)	Agent(s)
penicillin	penicillin
aminopenicillin	amoxicillin
	ampicillin
ureidopenicillin	piperacillin
carboxypenicillin	carbenicillin
	ticarcillin

β-lactamase-labile penicillins

Staphylococcus aureus
Staphylococcus lugdunensis
Moraxella catarrhalis
Haemophilus influenzae
Bacteroides fragilis

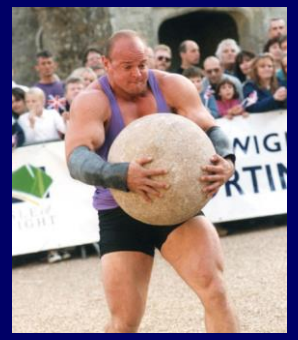
DRUG COMPANIES FIGHT BACK



sulbactam
tazobactam
clavulanic acid

CEPHEMS

Activity	Generation
Narrow spectrum	First
Expanded spectrum	Second
Broad spectrum	Third
Extended spectrum	Fourth
MRSA	Fifth

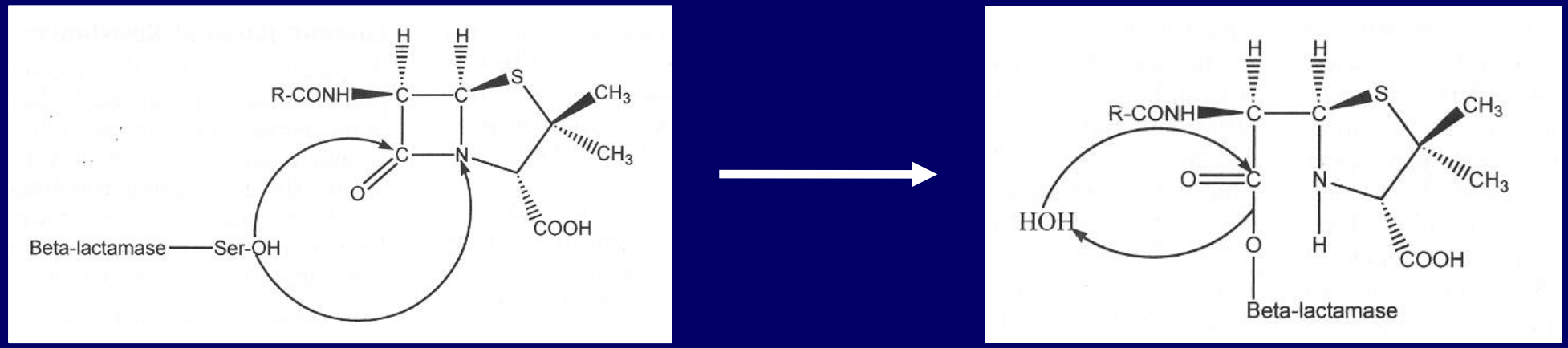


β-LACTAM RESISTANCE

- Mediated by β-lactamases

  >1000 individual enzymes have been reported

  Some are extended-spectrum β-lactamases (promiscuous)
  Some are chromosomal cephalosporinases (stay at home)
  Some are carbapenemases
  Some are metallo-β-lactamases



COMPANIES REALLY FIGHT BACK I

Table 2B-1
Pseudomonas aeruginosa
CLSI M02 and CLSI M07

β-LACTAM COMBINATION AGENTS								
(7) Organisms that test susceptible to the β-lactam agent alone are also considered susceptible to the β-lactam combination agent. However, organisms that test susceptible to the β-lactam combination agent cannot be assumed to be susceptible to the β-lactam agent alone. Similarly, organisms that test intermediate or resistant to the β-lactam agent alone may be susceptible to the β-lactam combination agent.								
Piperacillin-tazobactam	100/10 µg	≥ 22	18–21	≤ 17	≤ 16/4	32/4	≥ 64/4	**(8)** Breakpoints for intermediate are only to provide a buffer zone to prevent small uncontrolled technical factors from causing major discrepancies in interpretation.
Ceftazidime-avibactam	30/20 µg	≥ 21	–	≤ 20	≤ 8/4	–	≥ 16/4	
Ceftolozane-tazobactam	30/10 µg	≥ 21	17–20^	≤ 16	≤ 4/4	8/4^	≥ 16/4	
Imipenem-relebactam	10/25 µg	≥ 23	20–22^	≤ 19	≤ 2/4	4/4^	≥ 8/4	
Ticarcillin-clavulanate*	75/10 µg	≥ 24	16–23^	≤ 15	≤ 16/2	32/2–64/2^	≥ 128/2	

CLSI M100-Ed35, 2025

AAC
Journals.ASM.org

Antimicrobial Activity of Ceftolozane-Tazobactam Tested against *Enterobacteriaceae* and *Pseudomonas aeruginosa* with Various Resistance Patterns Isolated in U.S. Hospitals (2011-2012)

David J. Farrell,[a,b] Robert K. Flamm,[a] Helio S. Sader,[a,c] Ronald N. Jones[a,d]

JMI Laboratories, North Liberty, Iowa, USA[a]; Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada[b]; Division of Infectious Diseases, Federal University of São Paulo, São Paulo, SP, Brazil[c]; Tufts University School of Medicine, Boston, Massachusetts, USA[d]

P. aeruginosa resistance status (no. of isolates tested) and antimicrobial agent[a]	MIC_{50}	MIC_{90}	% susceptible[b]	% resistant[b]
All isolates (1,971)				
Ceftolozane/tazobactam	0.5	2	—[c]	—
Ceftazidime	2	32	82.9	13.7
Cefepime	4	16	82.4	8.6
Meropenem	0.5	8	80.3	13.9
Piperacillin-tazobactam	8	>64	76.8	13.7
Aztreonam	8	>16	68.5	19.2
Levofloxacin	0.5	>4	74.9	19.1
Gentamicin	≤1	8	89.2	7.7
Colistin	1	2	99.1	0.2
MDR (310)				
Ceftolozane/tazobactam	2	8	—	—
Ceftazidime	32	>32	22.6	60.6
Cefepime	16	>16	22.5	38.7
Meropenem	8	>8	19.4	64.5
Piperacillin-tazobactam	>64	>64	11.0	60.0
Aztreonam	>16	>16	9.0	69.0
Levofloxacin	>4	>4	15.2	70.6
Gentamicin	4	>8	53.5	36.5
Colistin	1	2	98.4	0.3
XDR (175)				
Ceftolozane/tazobactam	4	16	—	—
Ceftazidime	32	>32	9.1	73.7
Cefepime	>16	>16	10.9	52.0
Meropenem	8	>8	7.4	76.0
Piperacillin-tazobactam	>64	>64	2.3	74.9
Aztreonam	>16	>16	4.6	72.6
Levofloxacin	>4	>4	2.9	88.0
Gentamicin	8	>8	38.9	49.7
Colistin	1	2	97.7	0.6

[a] Abbreviations: MDR, multidrug resistant; XDR, extensively drug resistant (14).
[b] According to CLSI interpretive criteria (13).
[c] —, no published interpretive criteria.

Antimicrob Agents Chemother. 57:6305-6310; 2013

CEFTOLOZANE-TAZOBACTAM

Parameter	Description
a.k.a.	ZERBAXA
Indication	1. Hospital-acquired, ventilator-associated pneumonia 2. Complicated urinary tract infections (including pyelonephritis) 3. Complicated intraabdominal infections (when combined with metronidazole)
Mechanism of action	1. Forms irreversible complex with β-lactamase 2. Binds PBP-1b, -1c, and -3 of *P. aeruginosa* Binds PBP-3 of *E. coli* to inhibit cell wall synthesis
Activity rendered	Cidal
Route of administration	IV
Half-life	3.12 h → q8h
Excretion	Renal

CEFTOLOZANE-TAZOBACTAM

Parameter	Description
Spectrum of activity	*Pseudomonas aeruginosa* *Enterobacterales* (*E. coli*, *K. pneumoniae*, *K. oxytoca*, *E. cloacae*, *P. mirabilis, Serratia marcescens*) *Haemophilus influenzae* *Bacteroides fragilis* *Streptococcus anginosus* group Claims activity versus ESBL producers
Adverse effects	Hypersensitivity in penicillin-, cephem-, or penem-allergic patients *C. difficile* infection

CEFTOLOZANE-TAZOBACTAM

Organism	Method	Testing/ Reporting	Breakpoint Range
Enterobacterales	BMD, DD	Tier 4	full
Pseudomonas aeruginosa	BMD, DD	Tier 3	full
Haemophilus influenzae	BMD	Tier 4	susceptible only
Viridans group *Streptococcus*	BMD	Tier 4	full

CLSI M100-Ed35, 2025

COMPANIES REALLY FIGHT BACK II

Table 2A-1
Enterobacterales (excluding *Salmonella* and *Shigella* spp.)
CLSI M02 and CLSI M07

β-LACTAM COMBINATION AGENTS										
(9) Organisms that test susceptible to the β-lactam agent alone are also considered susceptible to the β-lactam combination agent. However, organisms that test susceptible to the β-lactam combination agent cannot be assumed to be susceptible to the β-lactam agent alone. Similarly, organisms that test SDD, intermediate, or resistant to the β-lactam agent alone may be susceptible to the β-lactam combination agent.										
Amoxicillin-clavulanate	20/10 µg	≥ 18	–	14–17^	≤ 13	≤ 8/4	–	16/8^	≥ 32/16	**(10)** Breakpoints when oral amoxicillin-clavulanate is used are only for therapy of uncomplicated UTIs or for completion of therapy for systemic infection.
Ampicillin-sulbactam	10/10 µg	≥ 15	–	12–14^	≤ 11	≤ 8/4	–	16/8^	≥ 32/16	
Ceftolozane-tazobactam	30/10 µg	≥ 22	–	19–21^	≤ 18	≤ 2/4	–	4/4^	≥ 8/4	
Ceftazidime-avibactam	30/20 µg	≥ 21	–	–	≤ 20	≤ 8/4	–	–	≥ 16/4	**(11)** Confirmatory MIC testing is indicated for isolates with zones of 20–22 mm to avoid reporting false-susceptible or false-resistant results.
Imipenem-relebactam	10/25 µg	≥ 25	–	21–24^	≤ 20	≤ 1/4	–	2/4^	≥ 4/4	**(12)** Breakpoints do not apply to the family Morganellaceae, which includes but is not limited to the genera *Morganella, Proteus,* and *Providencia.*
Meropenem-vaborbactam	20/10 µg	≥ 18	–	15–17^	≤ 14	≤ 4/8	–	8/8^	≥ 16/8	**(13)** Enterobacterales that harbor OXA-48–like enzymes may test susceptible to meropenem-vaborbactam but may not respond to meropenem-vaborbactam *in vivo.* If an OXA-48–like gene or enzyme is detected, suppress meropenem-vaborbactam or report as resistant.
Piperacillin-tazobactam	100/10 µg	≥ 25	21–24	–	≤ 20	≤ 8/4	16/4	–	≥ 32/4	
Ticarcillin-clavulanate*	75/10 µg	≥ 20	–	15–19^	≤ 14	≤ 16/2	–	32/2–64/2^	≥ 128/2	

CLSI M100-Ed35, 2025

PENEM CLASS

Parameter	Description
Mechanism of action	Bind to penicillin-binding proteins 1 and 2, causing cell elongation and eventual lysis
Activity rendered	Cidal
Route of administration	IV
Half-life	1-4 hrs → q8h or q24h
Excretion	Renal
Adverse effects	Nausea, vomiting, diarrhea 5%; drug fever, rash, urticaria 3%; seizures 1%; other reversible effects

PENEM CLASS

Parameter	Description
Spectrum of activity	Gram-positives (including penicillin-resist *S. pneumo*) Gram-negatives (including β-lactam- and aminoglycoside-resistant enterics, ESBL) Not effective versus MRSA, vancomycin-resistant *Enterococcus* spp., *Stenotrophomonas maltophilia* Most potent β-lactam versus anaerobes
Interesting stuff	Widest spectrum of antibacterial activity of currently-available antimicrobials; imipenem administered with cilastatin (a dehydropeptidase I inhibitor)

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

Antimicrobial Activity of Ceftazidime-Avibactam Tested against Multidrug-Resistant *Enterobacteriaceae* and *Pseudomonas aeruginosa* Isolates from U.S. Medical Centers, 2013 to 2016

Helio S. Sader, Mariana Castanheira, Dee Shortridge, Rodrigo E. Mendes, Robert K. Flamm

JMI Laboratories, North Liberty, Iowa, USA

Organism category and antimicrobial agent (no. of isolates tested)	MIC (μg/ml)		CLSI[b]		EUCAST	
	MIC_{50}	MIC_{90}	%S	%R	%S	%R
XDR (448)[d]						
Ceftazidime-avibactam	0.5	2	97.8	2.2*	97.8	2.2
Ceftriaxone	>8	>8	2.0	97.3	2.0	97.3
Ceftazidime	>32	>32	5.4	91.5	2.9	94.6
Cefepime	>16	>16	10.5	79.9	6.4	85.8
Piperacillin-tazobactam	>64	>64	7.1	83.7	6.5	92.9
Meropenem	8	>8	21.2	72.5	27.5	48.2
Levofloxacin	>4	>4	8.7	84.8	2.2	96.4
Gentamicin	8	>8	27.0	50.0	23.4	73.0
Amikacin	16	32	60.2	9.6	46.5	39.8
Tigecycline	0.5	4	90.0	0.2*	81.0	10.0
Colistin	≤0.5	>8			61.3	38.7
CRE (513)[e]						
Ceftazidime-avibactam	0.5	2	97.5	2.5*	97.5	2.5
Ceftriaxone	>8	>8	2.1	97.5	2.1	97.5
Ceftazidime	>32	>32	4.3	93.0	2.3	95.7
Cefepime	>16	>16	8.4	77.9	3.2	87.1
Piperacillin-tazobactam	>64	>64	3.1	91.2	2.7	96.9
Meropenem	>8	>8	2.7	89.7	10.3	52.4
Levofloxacin	>4	>4	23.4	72.9	15.0	81.3
Gentamicin	8	>8	49.5	33.9	44.4	50.5
Amikacin	8	32	68.2	7.0	51.5	31.8
Tigecycline	0.5	1	98.8	0.0*	90.3	1.2
Colistin	≤0.5	>8			79.1	20.9
P. aeruginosa						
All isolates (7,868)						
Ceftazidime-avibactam	2	4	97.1	2.9*	97.1	2.9
Ceftazidime	2	32	84.7	10.9	84.7	15.3
Cefepime	2	16	85.6	5.2	85.6	14.4
Piperacillin-tazobactam	4	64	81.0	9.4	81.0	19.0
Meropenem	0.5	8	81.3	12.8	81.3	6.8
Levofloxacin	0.5	>4	74.5	18.6	65.3	34.7
Gentamicin	2	8	87.0	8.4	87.0	13.0
Amikacin	4	8	96.5	1.9	91.8	3.5
Colistin	1	2	99.6	0.4	99.6	0.4

Antimicrob Agents Chemother. 61:e01045-17; 2017

CEFTAZIDIME-AVIBACTAM

Parameter	Description
a.k.a.	AVYCAZ
Indication	1. Hospital-acquired, ventilator-associated pneumonia 2. Complicated urinary tract infections (including pyelonephritis) 3. Complicated intraabdominal infections (when combined with metronidazole)
Mechanism of action	1. Inactivates β-lactamases 2. Binds essential penicillin-binding proteins
Activity rendered	Cidal
Route of administration	IV
Half-life	2.76 h → q8h
Excretion	Renal

CEFTAZIDIME-AVIBACTAM

Parameter	Description
Spectrum of activity	*Pseudomonas aeruginosa* *Enterobacterales* (*E. coli*, *K. pneumoniae*, *E. cloacae*, *P. mirabilis*, *C. freundii*) Claims activity versus ESBL producers
Adverse effects	Hypersensitivity in penicillin-, cephem-, or penem-allergic patients *C. difficile* infection CNS reactions, particularly in renal-impaired patients

CEFTAZIDIME-AVIBACTAM

Organism	Method	Testing/ Reporting	Breakpoint Range
Enterobacterales	BMD, DD	Tier 3	full
Pseudomonas aeruginosa	BMD, DD	Tier 3	full

CLSI M100-Ed35, 2025

EVERY SILVER LINING'S GOT A…

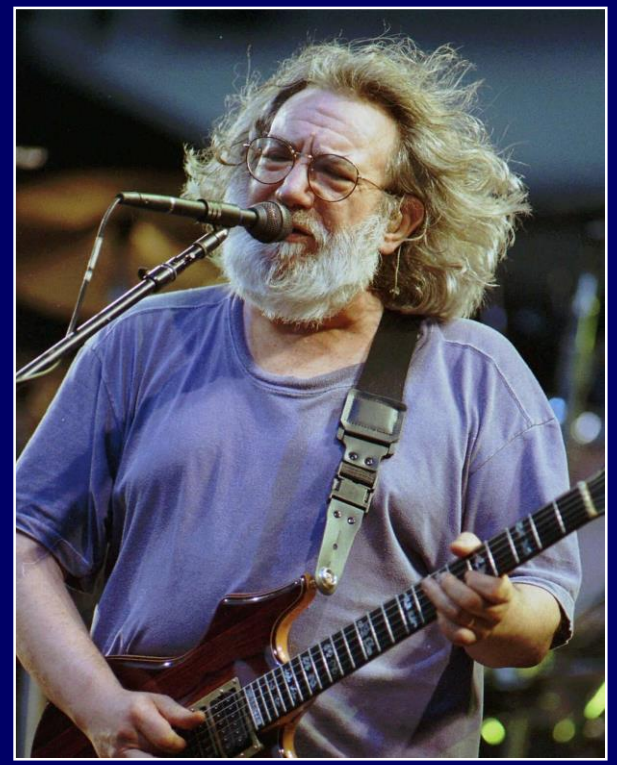
Sader *et al. BMC Pulmonary Medicine* (2025) 25:38
https://doi.org/10.1186/s12890-025-03500-8

BMC Pulmonary Medicine

RESEARCH **Open Access**

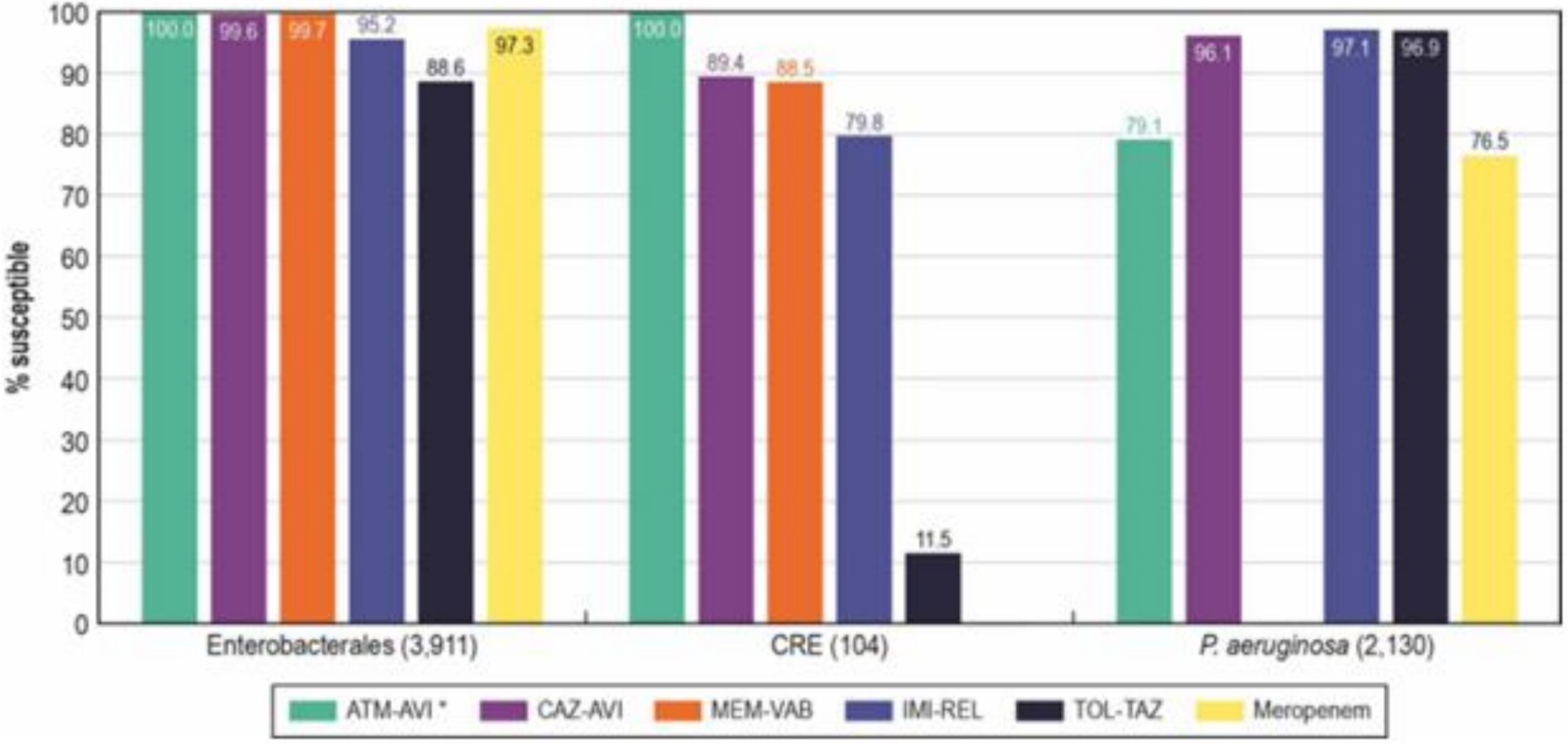
Activity of Aztreonam-avibactam and other β-lactamase inhibitor combinations against Gram-negative bacteria isolated from patients hospitalized with pneumonia in United States medical centers (2020–2022)

Helio S. Sader[1*], Rodrigo E. Mendes[1], S. J. Ryan Arends[1], Timothy B. Doyle[1] and Mariana Castanheira[1]



BMC Pulm Med. 25:38; 2025

…TOUCH OF GRAY



BMC Pulm Med. 25:38; 2025

PENEM RESISTANCE

- Antecedent ESBL or ampC + alteration of porin channels in cell wall, reducing permeability (CRE)
- Carbapenemase production (CPE…and CRE)

  Serine carbapenemases (class A β-lactamase)
  Metallo-β-lactamase (class B β-lactamase)
  Oxacillinase (class D β-lactamase)

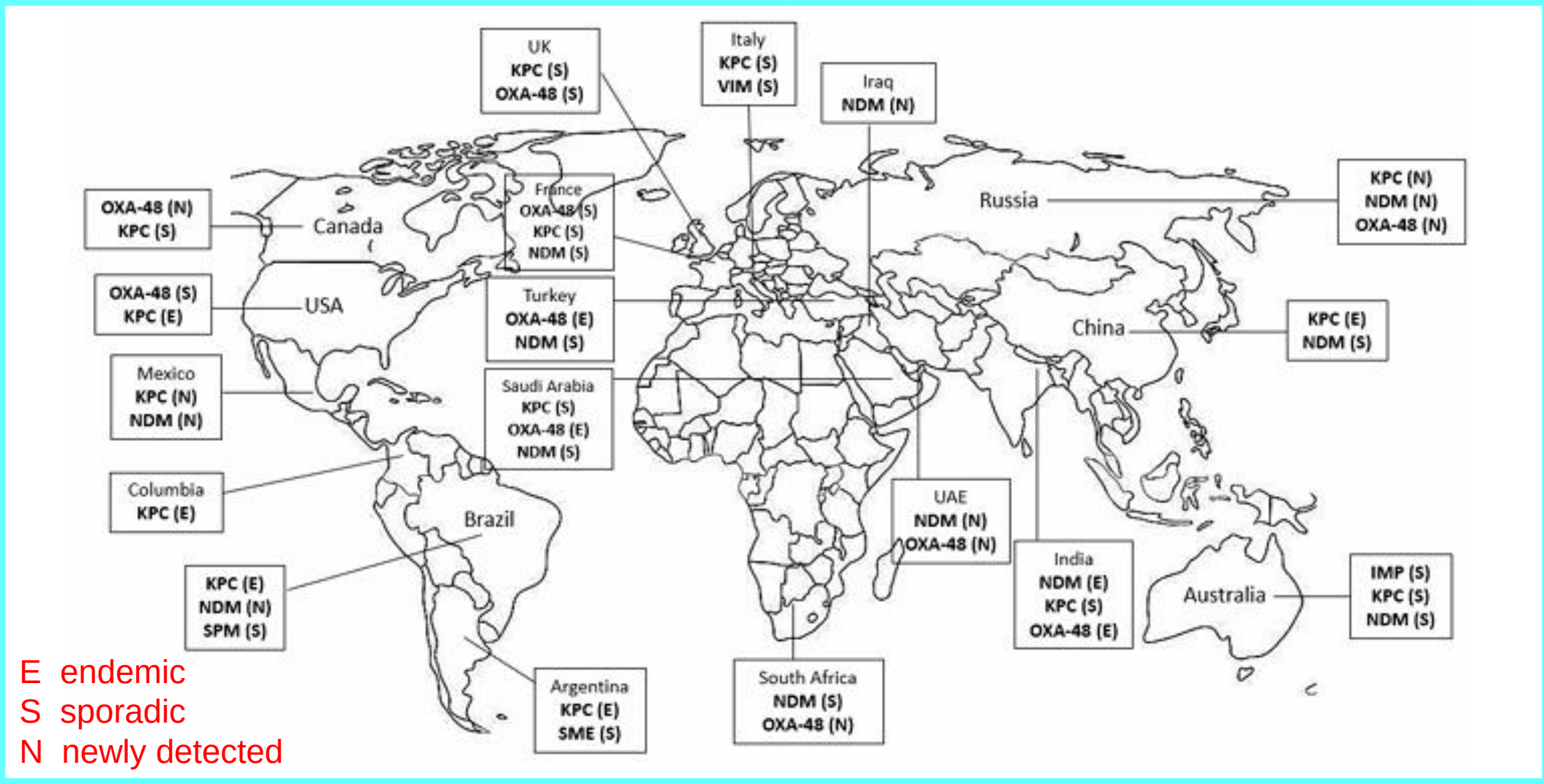
- CREs and CPEs commonly carry other resistance determinants

Infect Control Hosp Epidemiol. 29:1107-1109; 2008

AMBLER CARBAPENEMASE GROUPS

Group	Examples	Sample targets of hydrolysis	Doesn't touch	Inhibited by
A	KPC IMI SME	penicillins 1°, 2° cephems aztreonam carbapenems	cephamycins	clavulanic acid tazobactam
B	NDM IMP VIM	penicillins 1°, 2° cephems carbapenems	aztreonam	EDTA (chelators)
D	OXA	higher penicillins higher cephems		none of the above

Antibiotics 9:186; 2020



Antibiotics 9:186; 2020

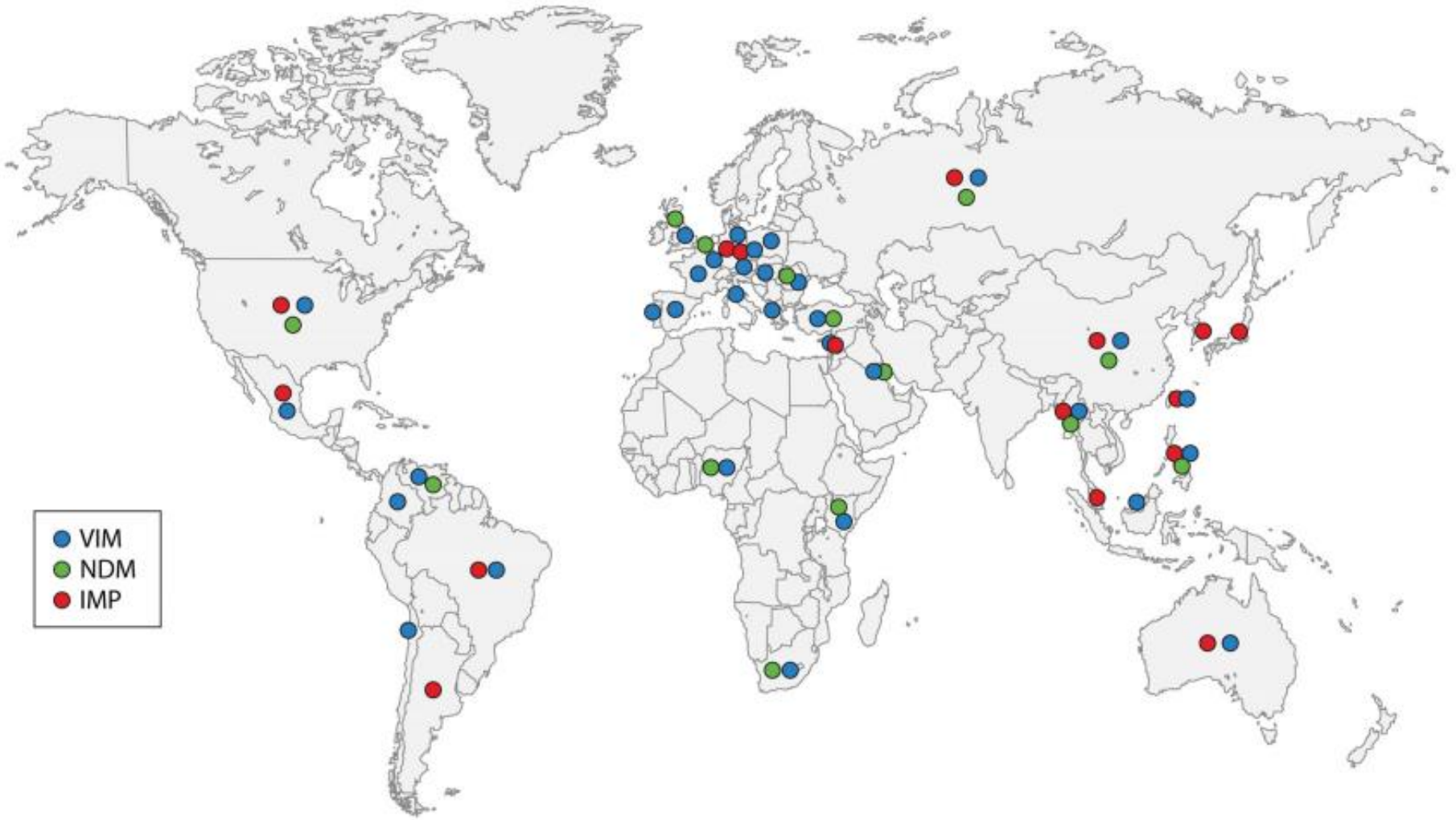
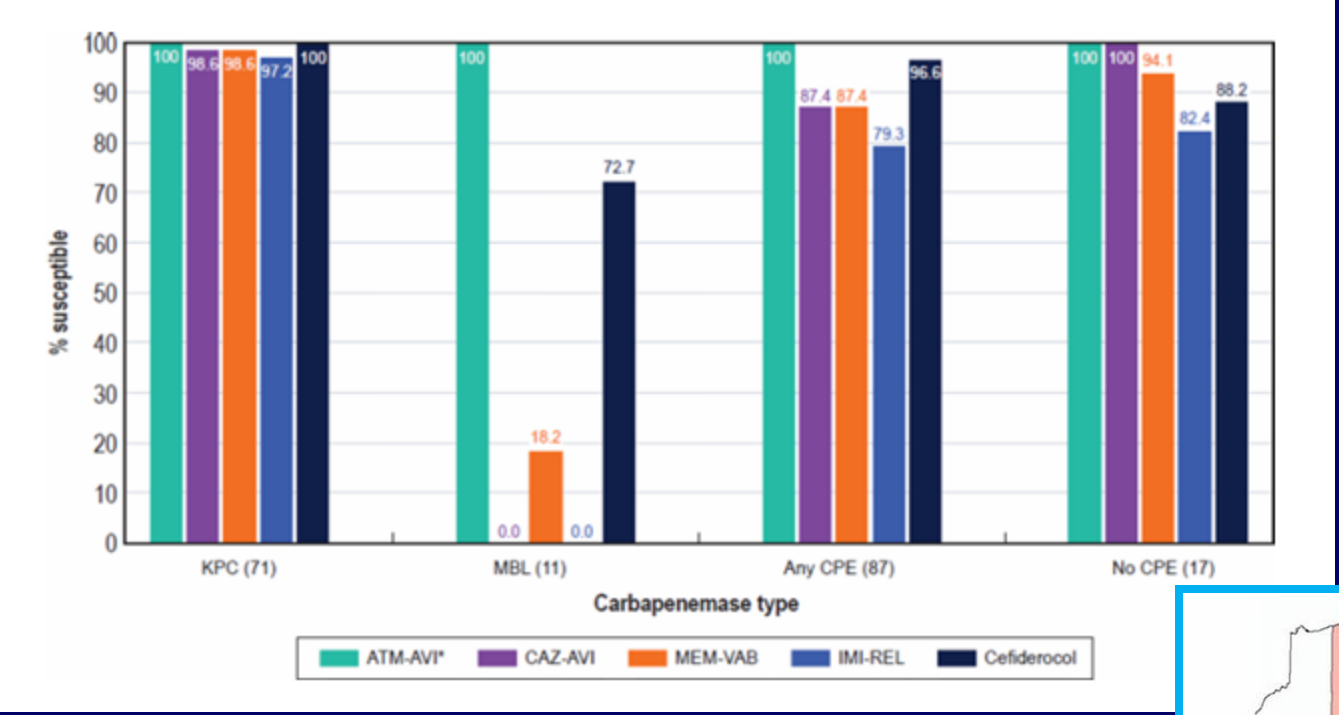
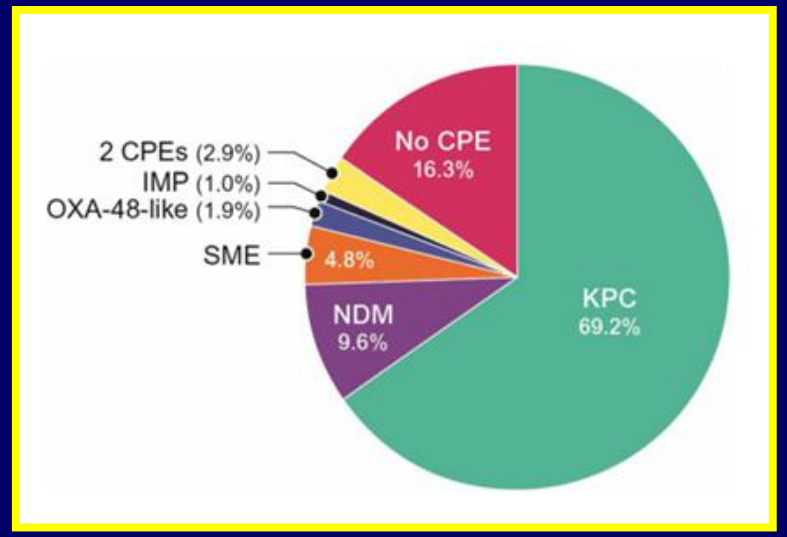


FIG 5 Global distribution of metallo-β-lactamase-positive *Enterobacteriaceae* and *P. aeruginosa*, including NDM-type enzymes collected from 2012 to 2014 from surveillance. (Republished from reference 287).

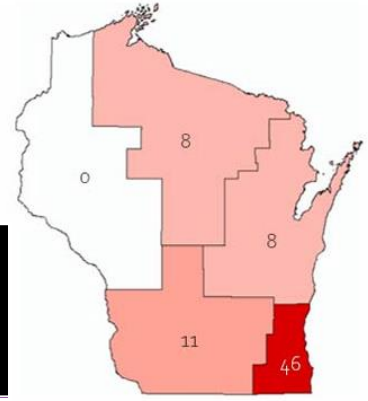
Clin Microbiol Rev. 33:e00047-19; 2020

AN OPTION FOR SOME

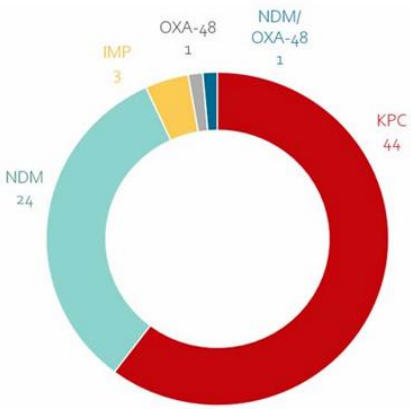




Laboratory-Based Surveillance Plan 2024-2025



Carbapenamase detections in CRE isolates, by region of the state.



Carbapenamase genes detected in CRE isolates by AR-targeted RT-PCR.

BMC Pulm Med. 25:38; 2025

AZTREONAM-AVIBACTAM

Parameter	Description
a.k.a.	EMBLAVEO
Indication	1. Complicated intraabdominal infections (when combined with metronidazole)
Mechanism of action	1. Inactivates β-lactamases 2. Binds essential penicillin-binding proteins
Activity rendered	Cidal
Route of administration	IV
Half-life	2.03 h → q8h
Excretion	Renal

AZTREONAM-AVIBACTAM

Parameter	Description
Spectrum of activity	*Enterobacterales* (*E. coli*, *K. pneumoniae*, *K. oxytoca*, *E. cloacae*, *Serratia marcescens*, *C. freundii*)
Adverse effects	Hypersensitivity *C. difficile* infection Elevated serum transaminases Epidermal necrolysis in patients undergoing bone marrow transplant

AZTREONAM-AVIBACTAM

Organism	Method	Testing/ Reporting	Breakpoint Range

CLSI M100-Ed35, 2025

…TOUCH OF GRAY

Table 5
Activity of aztreonam and aztreonam/avibactam (MIC in mg/L) against different enzyme variants and combinations for all Enterobacterales, 2019.

All Enterobacterales[a] (N = 18 713)	Drug	n	MIC (mg/L)			%S CLSI	%S EUCAST[b]
			MIC Range	MIC_{50}	MIC_{90}		
MBL positive[c]	Aztreonam	462	0.015–256	128	256	14.7	12.6
	Aztreonam/avibactam[d]		0.015–16	0.12	0.5	99.6	
IMP[e]	Aztreonam	6	0.25–128	64	128	33.3	33.3
	Aztreonam/avibactam[d]		0.03–2	0.25	2	100.0	
VIM[f]	Aztreonam	49	0.06–256	64	128	18.4	18.4
	Aztreonam/avibactam[d]		0.015–2	0.12	0.5	100.0	
NDM[g]	Aztreonam	408	0.015–256	128	256	14.2	14.2
	Aztreonam/avibactam[d]		0.015–16	0.12	0.5	99.5	
NDM-1	Aztreonam	270	0.015–256	128	256	14.4	14.4
	Aztreonam/avibactam[d]		0.015–4	0.12	0.5	100.0	
NDM-5	Aztreonam	113	0.015–256	128	256	13.3	13.3
	Aztreonam/avibactam[d]		0.015–16	0.25	4	98.2	
NDM-7	Aztreonam	17	0.03–256	128	256	23.5	23.5
	Aztreonam/avibactam[d]		0.03–0.5	0.12	0.5	100.0	
IMP+VIM	Aztreonam	55	0.06–256	64	128	20	20
	Aztreonam/avibactam[d]		0.015–2	0.12	0.5	100.0	
IMP+NDM	Aztreonam	414	0.015–256	128	256	14.5	14.5
	Aztreonam/avibactam[d]		0.015–16	0.12	0.5	99.5	
NDM+VIM	Aztreonam	456	0.015–256	128	256	14.5	14.5
	Aztreonam/avibactam[d]		0.015–16	0.12	0.5	99.6	
KPC positive[h]	Aztreonam	368	2–256	256	256	2.5	2.5
	Aztreonam/avibactam[d]		0.015–4	0.25	0.5	100.0	
OXA positive[i]	Aztreonam	461	0.06–256	128	256	9.3	9.3
	Aztreonam/avibactam[d]		0.015–16	0.25	0.5	99.8	
KPC+MBL positive	Aztreonam	820	0.015–256	128	256	9.4	9.4
	Aztreonam/avibactam[d]		0.015–16	0.25	0.5	99.8	
OXA+MBL positive	Aztreonam	843	0.015–256	128	256	12.3	12.3
	Aztreonam/avibactam[d]		0.015–16	0.25	0.5	99.6	
KPC+OXA+MBL positive	Aztreonam	1197	0.015–256	128	256	9.4	9.4
	Aztreonam/avibactam[d]		0.015–16	0.25	0.5	99.8	

J Glob Antimicrob Resist. 30:214-221; 2022

THIS GETS COMPLICATED

- NDM isolates frequently harbor other β-lactamases

  Able to hydrolyze aztreonam
  Inhibited by avibactam

- Aztreonam and ceftazidime-avibactam (ATM-CZA)

  Clinical efficacy against multi-drug- and

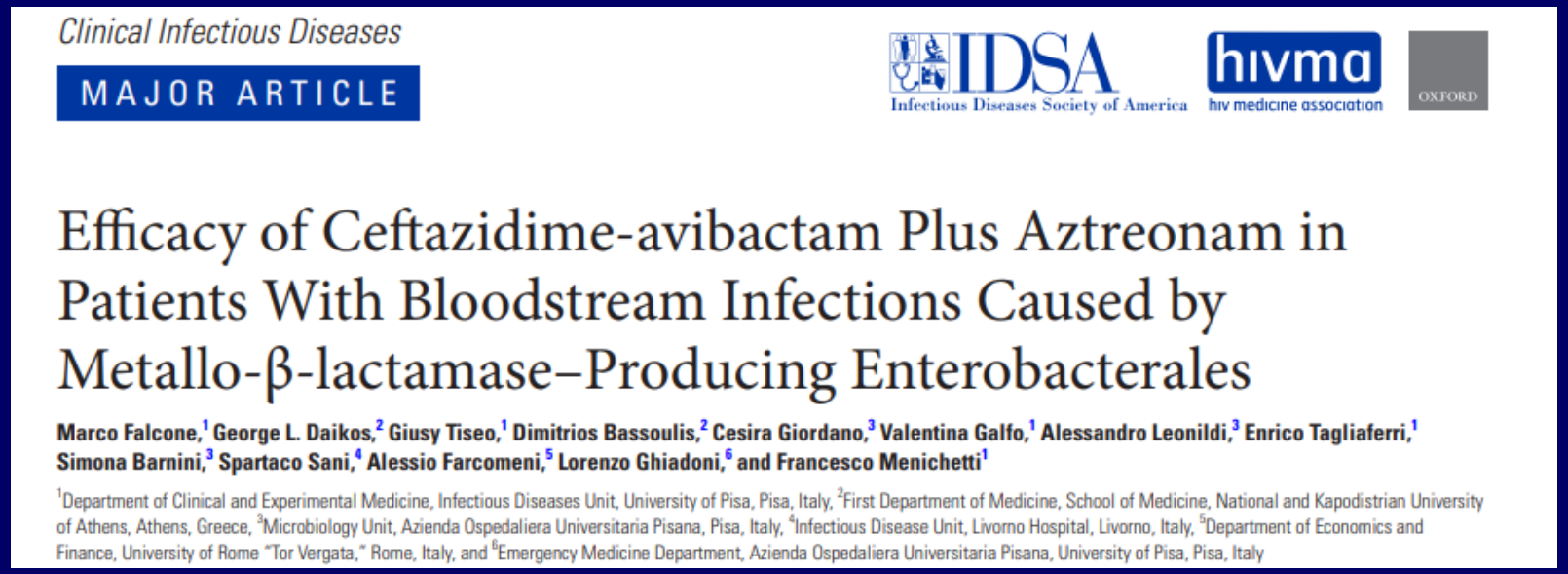
  resistant to three or more classes

  extensively drug-resistant

  resistant to all but one or two classes

  Enterobacterales (next two slides)

J Clin Microbiol. 61:e0164722; 2023



Clinical Infectious Diseases

MAJOR ARTICLE

IDSA Infectious Diseases Society of America | hivma hiv medicine association | OXFORD

Efficacy of Ceftazidime-avibactam Plus Aztreonam in Patients With Bloodstream Infections Caused by Metallo-β-lactamase–Producing Enterobacterales

Marco Falcone,[1] George L. Daikos,[2] Giusy Tiseo,[1] Dimitrios Bassoulis,[2] Cesira Giordano,[3] Valentina Galfo,[1] Alessandro Leonildi,[3] Enrico Tagliaferri,[1] Simona Barnini,[3] Spartaco Sani,[4] Alessio Farcomeni,[5] Lorenzo Ghiadoni,[6] and Francesco Menichetti[1]

[1]Department of Clinical and Experimental Medicine, Infectious Diseases Unit, University of Pisa, Pisa, Italy, [2]First Department of Medicine, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece, [3]Microbiology Unit, Azienda Ospedaliera Universitaria Pisana, Pisa, Italy, [4]Infectious Disease Unit, Livorno Hospital, Livorno, Italy, [5]Department of Economics and Finance, University of Rome "Tor Vergata," Rome, Italy, and [6]Emergency Medicine Department, Azienda Ospedaliera Universitaria Pisana, University of Pisa, Pisa, Italy

- 102 bloodstream infections

  82 NDM; 20 VIM (carbapenemase)
  93 *Klebsiella pneumoniae*, 5 *Enterobacter* spp.

- 52 received ATM-CZA
  50 received other active antibiotics (OAA)

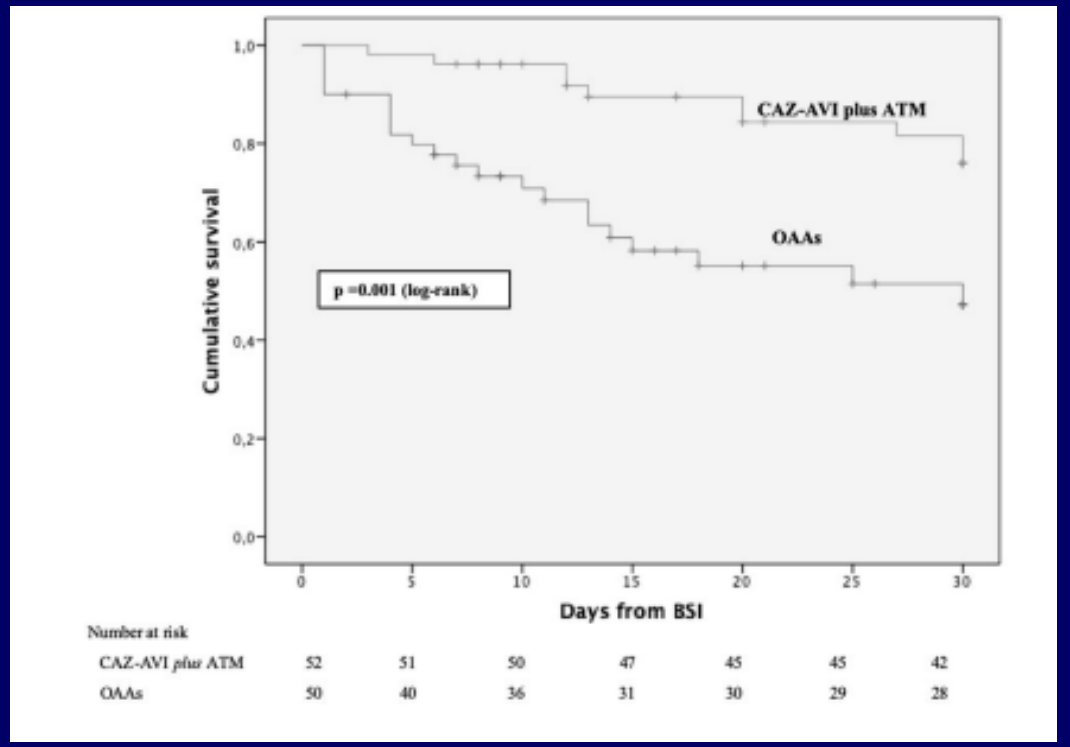
  27 with colistin

Clin Infect Dis. 72:1871-1878; 2021

CLINICAL EFFICACY

Table 2. Targeted Antibiotic Regimens Administered in 102 Bloodstream Infections Due to Metallo-β-Lactamase–Producing Enterobacterales

Antibiotic Regimen	No. (%) (N = 102)	Mortality, No. (%)
CAZ-AVI + ATM[a]	52 (51)	10/52 (19.2)
OAAs		
Colistin-containing regimens	27 (26.5)	16/27 (59.3)
Colistin + fosfomycin + tigecycline	7	6/7
Colistin + fosfomycin	7	5/7
Colistin + meropenem	5	3/5
Colistin + ATM ± piperacillin-tazobactam	4	1/4
Colistin + gentamicin	1	0/1
Colistin + cotrimoxazole	1	0/1
Colistin alone	2	1/2
Regimens not containing colistin	23 (22.5)	6/23 (26.1)
Tigecycline + aminoglycosides	8	2/8
Fosfomycin + aminoglycosides	5	0/5
Tigecycline + fosfomycin	2	2/2
Tigecycline + meropenem	1	0/1
ATM + aminoglycosides	4	1/4
ATM + fosfomycin	1	0/1
ATM alone	2	1/2



↓ 30d mortality rate $P = 0.007$

↓ d14 clinical failure $P = 0.002$

shorter length of stay $P = 0.007$

Clin Infect Dis. 72:1871-1878; 2021

Table 3D. Aztreonam Plus Ceftazidime-Avibactam Broth Disk Elution Method[1]

Due to limited therapeutic options, there may be a clinical need to assess the *in vitro* activity of the combination of aztreonam and ceftazidime-avibactam to guide therapeutic management of multidrug-resistant gram-negative bacterial infections, especially those caused by MBL producers.

The aztreonam plus ceftazidime-avibactam broth disk elution method was established with limited disk and/or media manufacturers and is considered provisional until additional data are evaluated by CLSI and shown to meet CLSI M23[2] guidance.

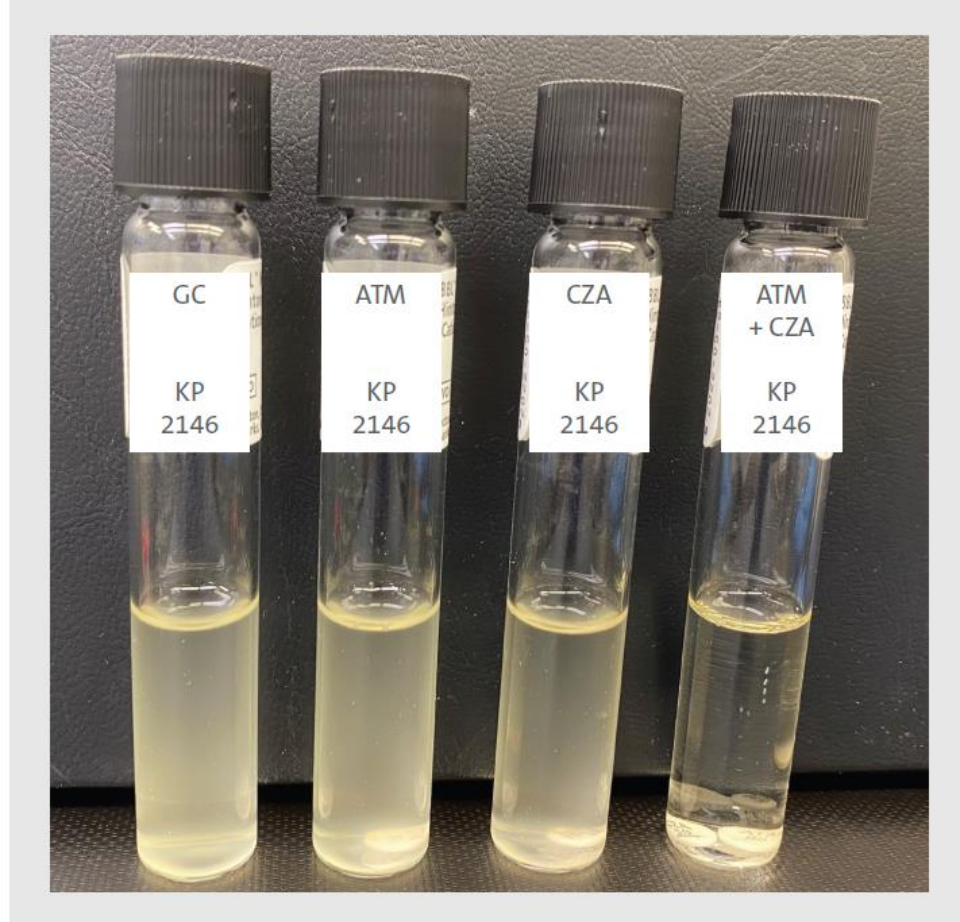
NOTE 1: Manufacturer-related issues were observed with different combinations of antimicrobial disks and CAMHB when the aztreonam plus ceftazidime-avibactam broth disk elution method was performed. QC of the method must be performed with every new lot or shipment of reagents to ensure the accuracy of results.

NOTE 2: Information in boldface type is new or modified since the previous edition.

Test	Aztreonam Plus Ceftazidime-Avibactam Broth Disk Elution
Organism group	Enterobacterales and *Stenotrophomonas maltophilia*
When to perform this test	Testing multidrug-resistant isolates, especially MBL producers
Test method	Tube dilution using aztreonam and ceftazidime-avibactam disks as the antimicrobial source
Medium	CAMHB (5-mL tubes)
Antimicrobial concentration	30-µg aztreonam disks 30/20-µg ceftazidime-avibactam disks Final concentration: 6 µg/mL aztreonam, 6 µg/mL ceftazidime, 4 µg/mL avibactam
Inoculum	1. Using a loop or swab, pick 3–5 colonies from a fresh (18–24 hours) nonselective agar plate and transfer to sterile saline (4–5 mL). 2. Adjust turbidity to equivalent of a 0.5 McFarland turbidity standard.

CLSI M100-Ed34, 2024

BROTH DISK ELUTION METHOD

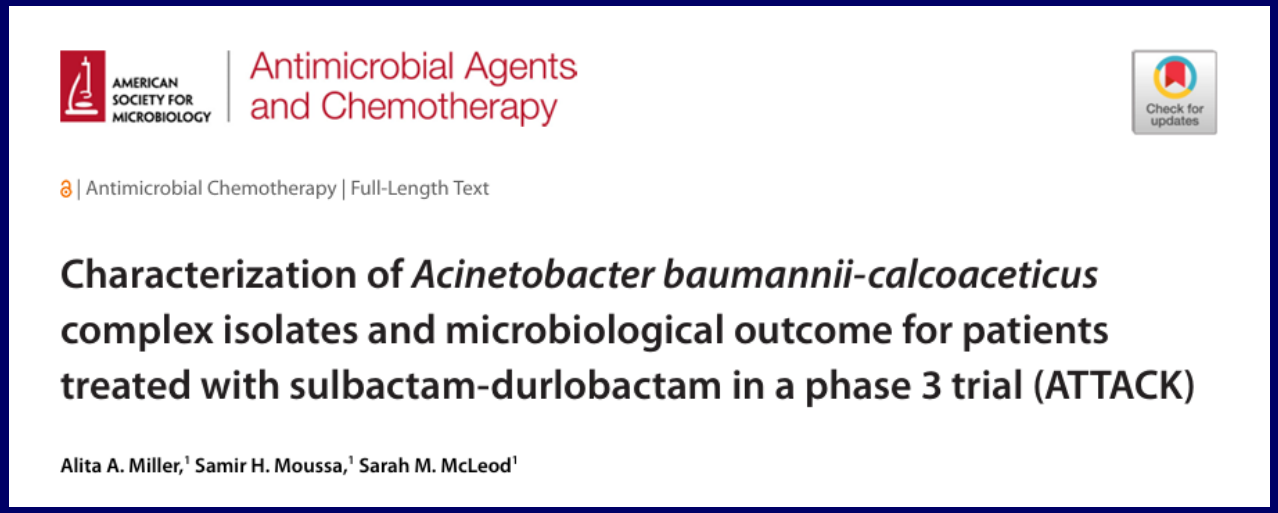


Klebsiella pneumoniae ATCC BAA-2146

not susceptible to ATM or CZA; susceptible to ATM-CZA

CLSI M100-Ed34, 2024

ONE LAST THING



sulbactam-durlobactam

sulbactam with intrinsic activity vs. *Acinetobacter*
durlobactam active vs. A, C, D serine β-lactamases

CLSI Tier 3; DD and BMD (≤4, 8, ≥16)

Antimicrob Agents Chemother. 68:e0169823; 2024

SULBACTAM-DURLOBACTAM

Antibacterial agent	MIC (µg/mL)			% NS (CLSI)
	Range	MIC_{50}	MIC_{90}	
Amikacin	1 to >64	>64	>64	85
Cefepime	1 to >16	>16	>16	95
Cefoperazone-sulbactam, 2:1	1 to >32	32	>32	NA
Colistin	≤0.25 to >8	0.5	>8	17[b]
Imipenem	0.12 to >8	>8	>8	96
Meropenem	0.06 to >8	>8	>8	96
Levofloxacin	0.06 to >4	>4	>4	96
Minocycline	≤0.12 to >16	4	16	43
Tigecycline	0.06 to >4	1	2	NA
Sulbactam	1 to >64	32	>64	NA
Sulbactam-durlobactam	0.25–16	2	4	4.6

Category	ABC baseline isolates, *N* (%)	SUL-DUR MIC range (µg/mL)	SUL-DUR $MIC_{50/90}$ (µg/mL)
ALL	175 (100)	0.25–16	2/4
CARB-R	168 (96)	0.5–16	2/4
MDR	168 (96)	0.5–16	2/4
XDR	148 (85)	0.5–16	2/4
PDR	26 (15)	1–8	2/4

Antimicrob Agents Chemother. 68:e0169823; 2024

SULBACTAM-DURLOBACTAM

		SUL-DUR MIC of baseline ABC (μg/mL)			
	Total, N (%)	0.5	1	2	4
All evaluable patients who received SUL-DUR[a]					
Number of patients	87	5	28	43	11
(Presumed) Eradication	63 (72%)	3 (60%)	19 (68%)	32 (75%)	9 (82%)
(Presumed) Persistence	18 (21%)	2 (40%)	5 (18%)	10 (23%)	1 (9%)
Indeterminate	6 (7%)	0	4 (14%)	1 (2%)	1 (9%)

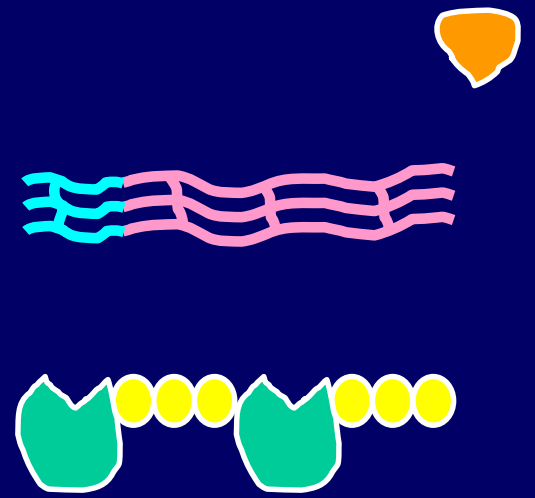
Antimicrob Agents Chemother. 68:e0169823; 2024

19% mortality in serious infections (including pneumonia)
32% mortality for colistin in randomized control trial

Lancet Infect Dis. 23:1072-1084; 2023

β-LACTAM RESISTANCE

- Mediated by penicillin-binding proteins
  - Penicillin-binding protein overexpression
    - 10-fold more PBP3 in *E. coli* than PBP2
  - Generation of point mutations
    - PBP5 of *E. faecalis* with ↓ affinity for penicillin
  - Acquisition of foreign PBP
    - MRSA
  - Recombination with foreign DNA
    - *S. pneumoniae*



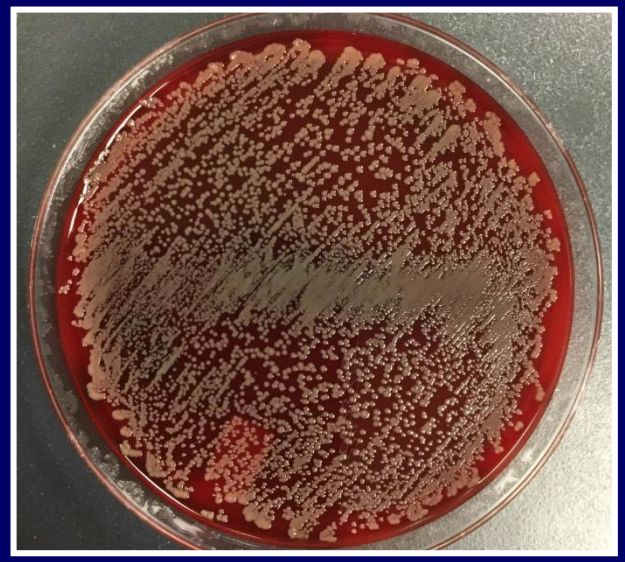
PENICILLIN CLASS

Subclass (if appropriate)	Agent(s)
penicillin	penicillin
aminopenicillin	amoxicillin
	ampicillin
ureidopenicillin	piperacillin
carboxypenicillin	carbenicillin
	ticarcillin
β-lactamase-stable penicillins	dicloxacillin
	methicillin
	nafcillin
	oxacillin

cefoxitin is a better *in vitro* inducer of *mecA* activity than oxacillin

ACQUISITION OF FOREIGN PBP

- *mecA* transcribed, translated into PBP2a
- Origin of *mecA* may be *Staphylococcus sciuri*



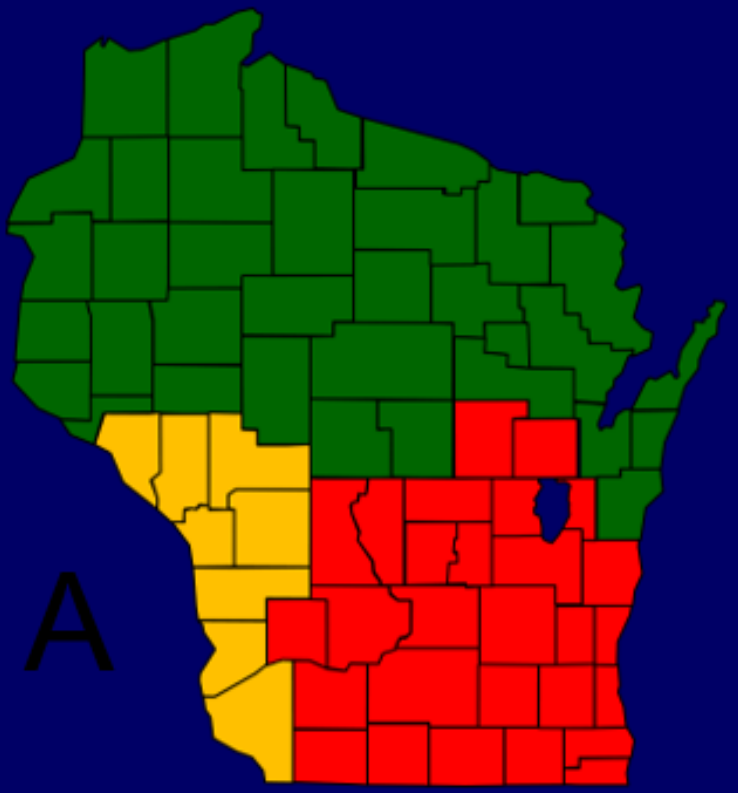
- *mecA* expression under influence of several regulatory genes
- Constituent of mobile SCC*mec* (staphylococcal cassette chromosome)

MRSA MECHANISM

- PBP2a has low affinity for

  Penicillins
  Carbapenems
  Majority of cephems

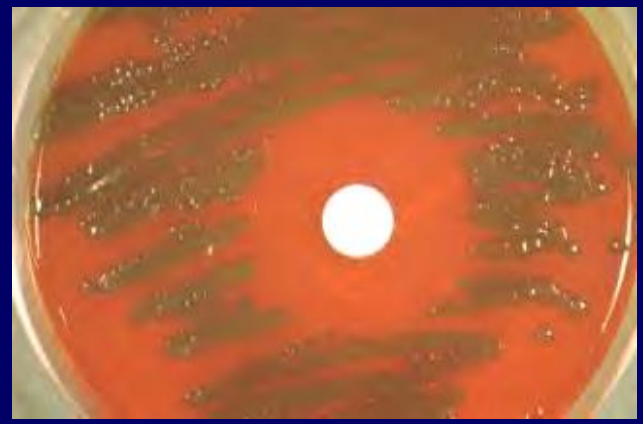
- While β-lactams bind to other PBP, PBP2a assumes peptidoglycan synthesis role

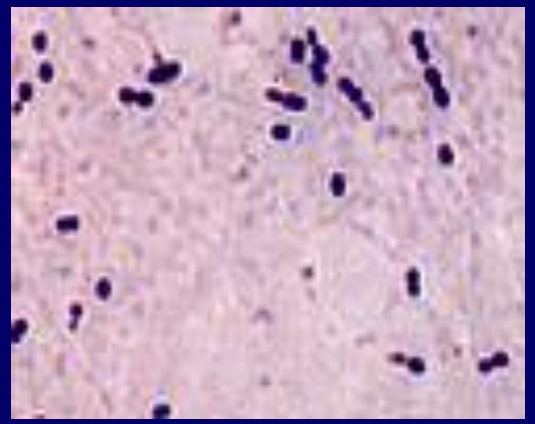


Clin Med Res. 17:72-81; 2019

RECOMBINATION W/ FOREIGN DNA

- PBP of less-susceptible species (viridans group *Streptococcus*) recombine with native species (*Streptococcus pneumoniae*)
- Organisms capable of uptake of “naked” DNA
- Highly-resistant *S. pneumoniae* implies more than one *pbp* being modified

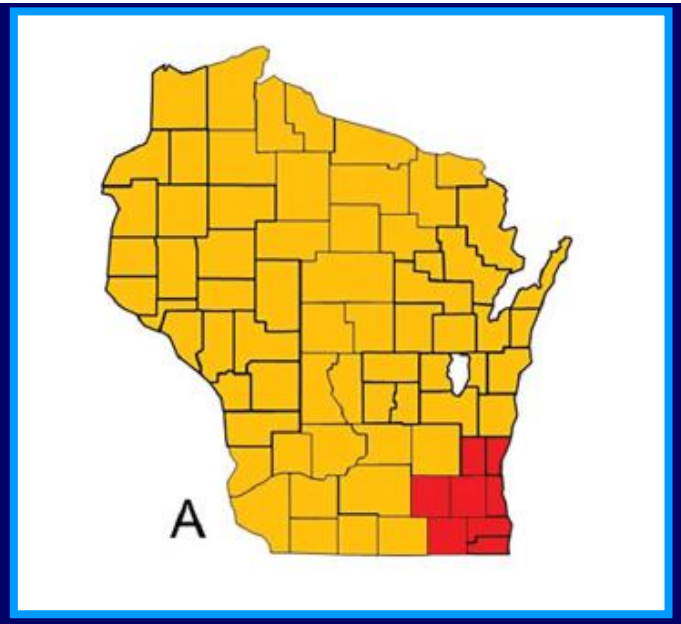




WISCONSIN DATA

Table 1: Antimicrobial susceptibility profiles for *Streptococcus pneumoniae* non-invasive and invasive isolates, Wisconsin 2016-2020.

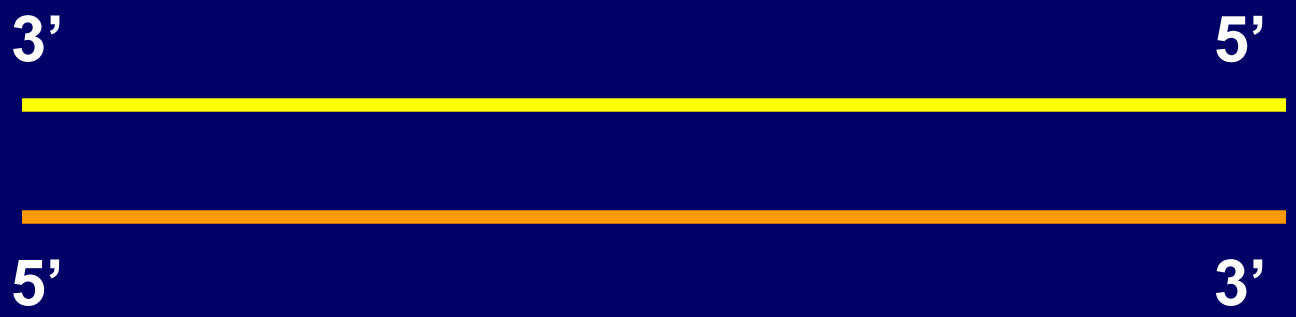
	Non-invasive		Invasive	
Antimicrobial Agent	n	Percentage Susceptible	n	Percentage Susceptible
Penicillin oral/CSF[a]	354	73.7	1070	78.7[b]
Penicillin non-CSF[a]	354	97.5	1020	99.4[c]
Ceftriaxone CSF[d]	354	93.8	1070	93.0
Ceftriaxone non-CSF[d]	354	97.7	1070	99.1[b]



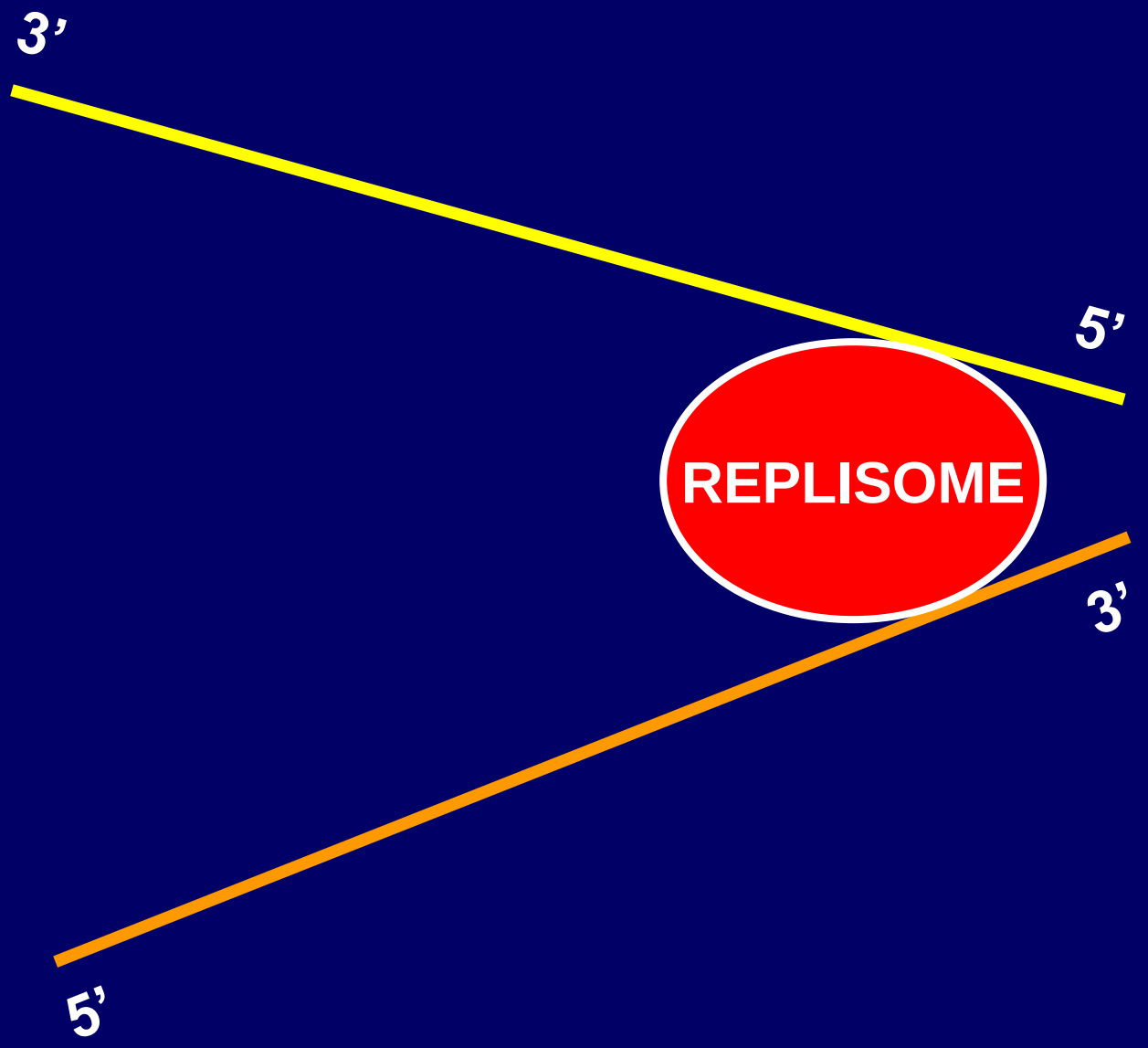
Clin Med Res. 20:185-194; 2022

Follow-up Non-β-lactam Resistance

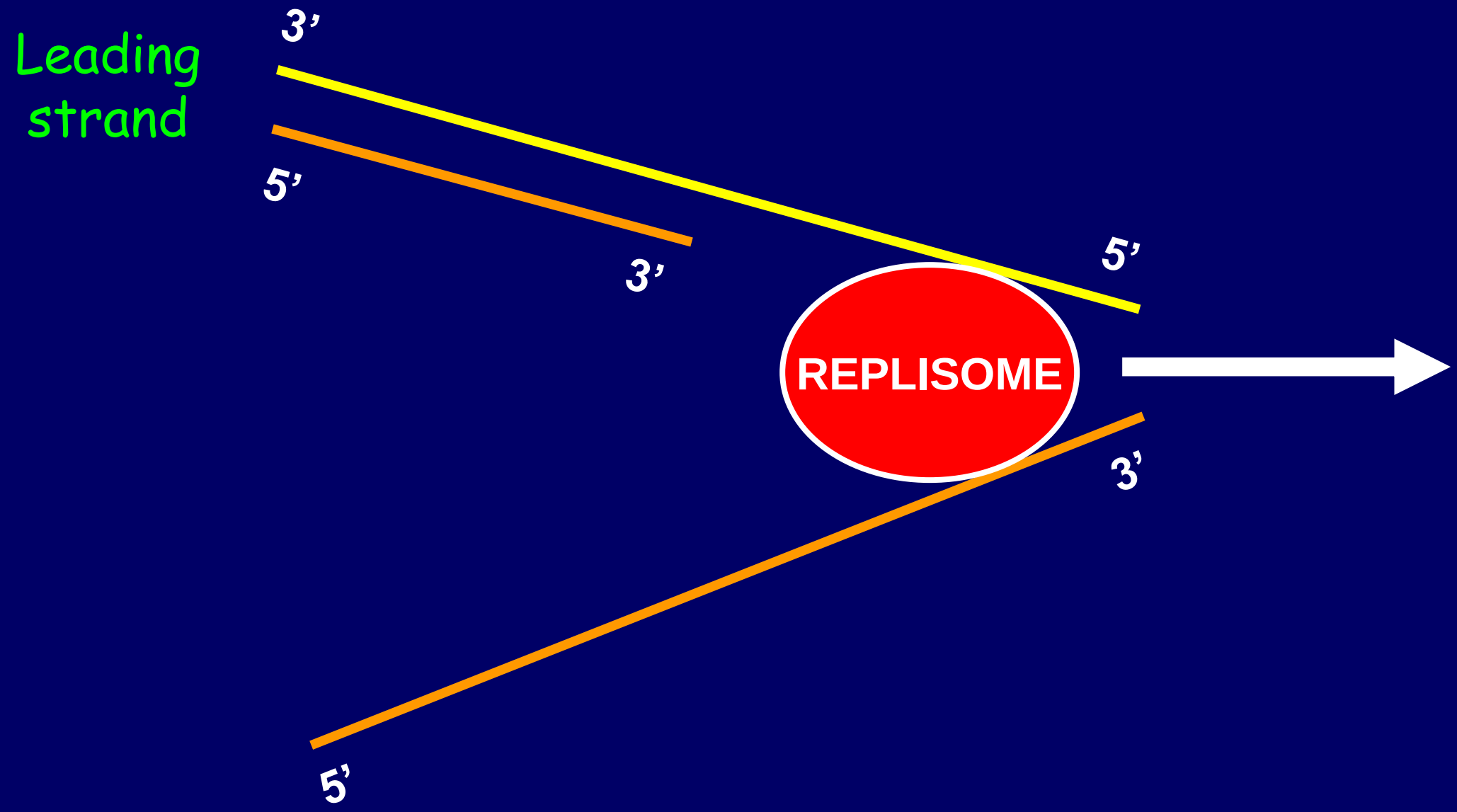
DNA REPLICATION



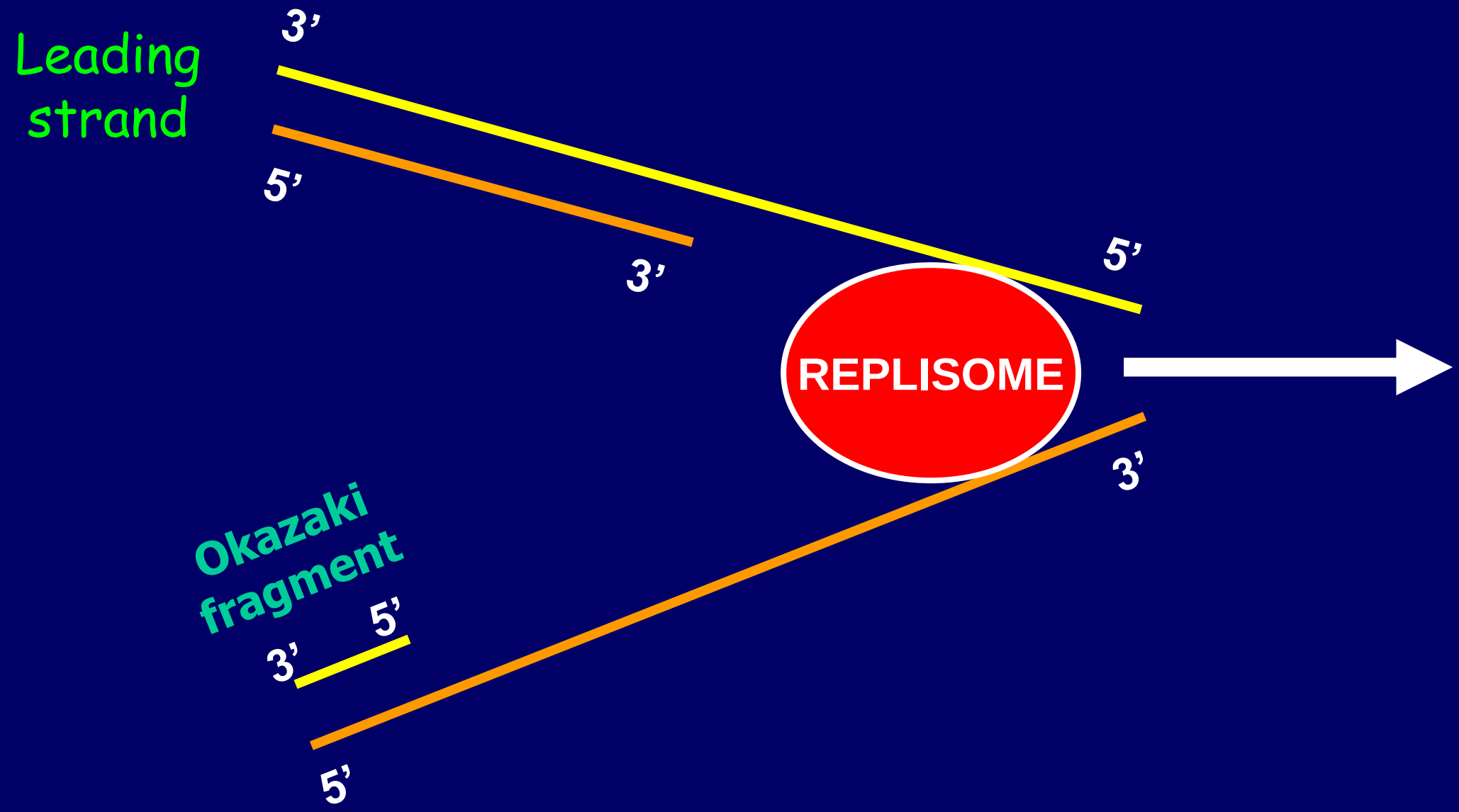
DNA REPLICATION



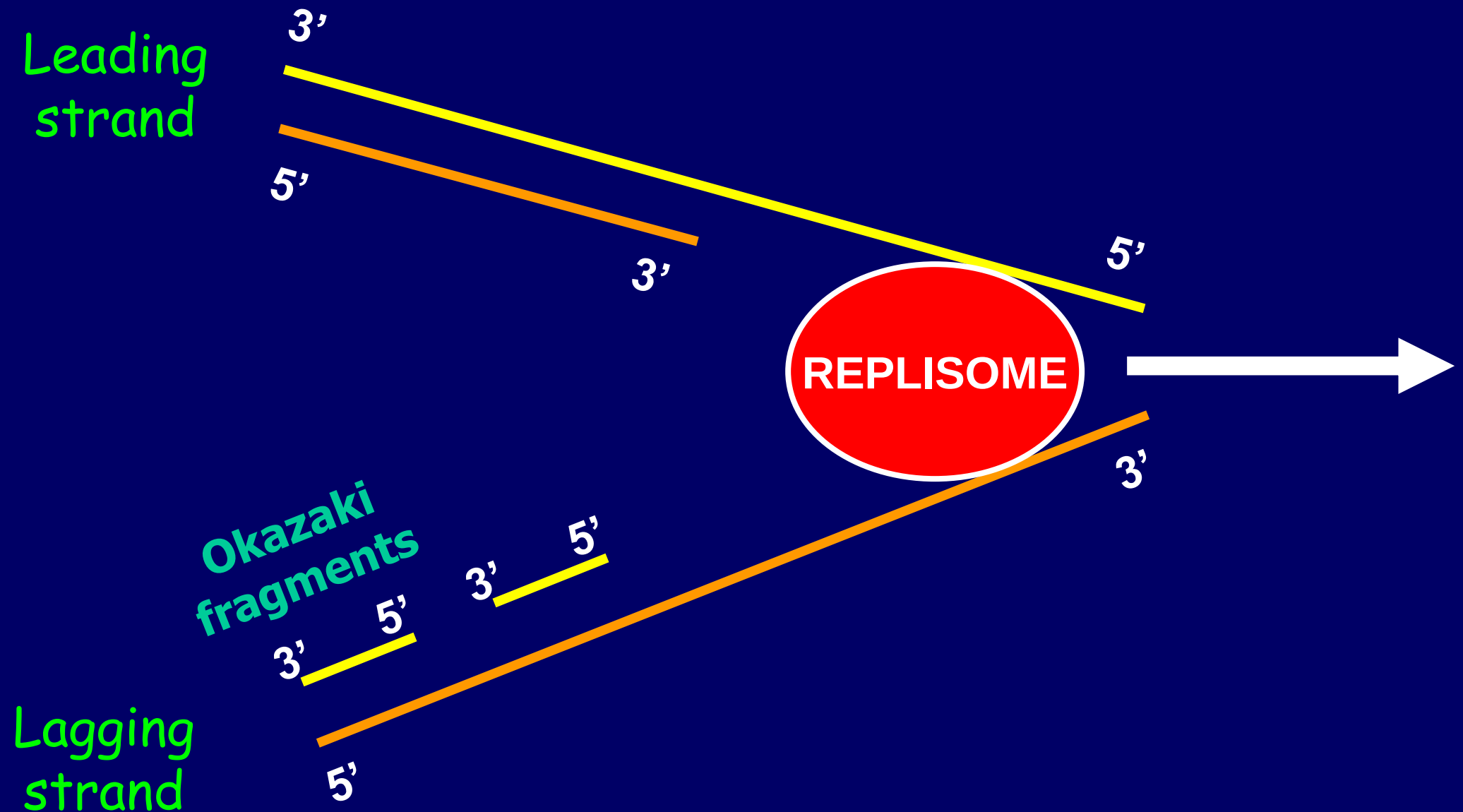
DNA REPLICATION



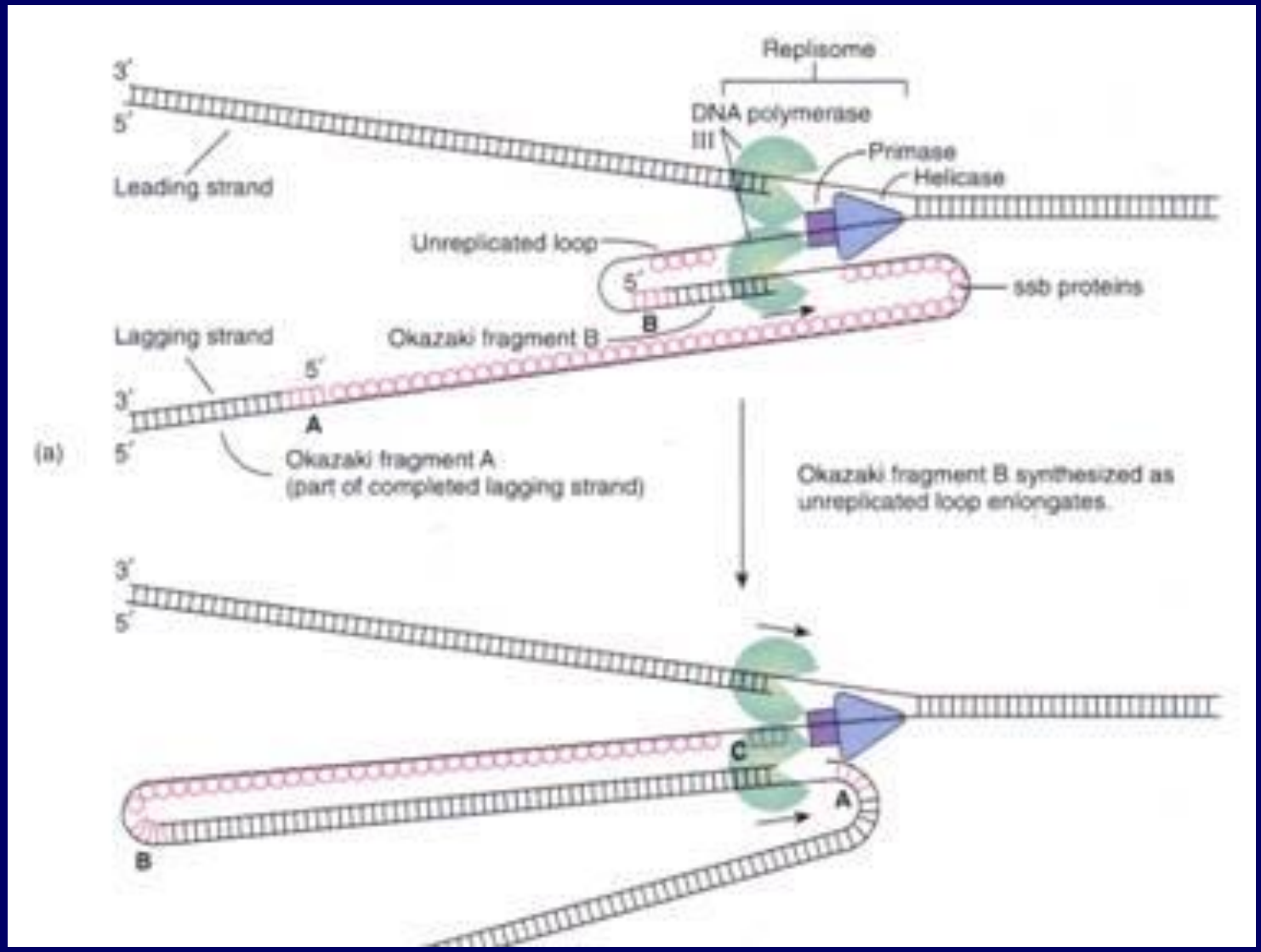
DNA REPLICATION



DNA REPLICATION



DNA REPLICATION



RELAXING/RECOVERY ENZYMES

- DNA topoisomerase IV (primarily Gram-positive)

parC ⟶ Two C subunits
parE ⟶ Two E subunits

- DNA gyrase (primary target in Gram-negative)

gyrA ⟶ Two GyrA subunits
gyrB ⟶ Two GyrB subunits

FLUOROQUINOLONE RESISTANCE

- Alterations in target enzymes

  Point mutations @ Ser83 and Asp87 for GyrA
  Ser79 and Asp83 for ParC

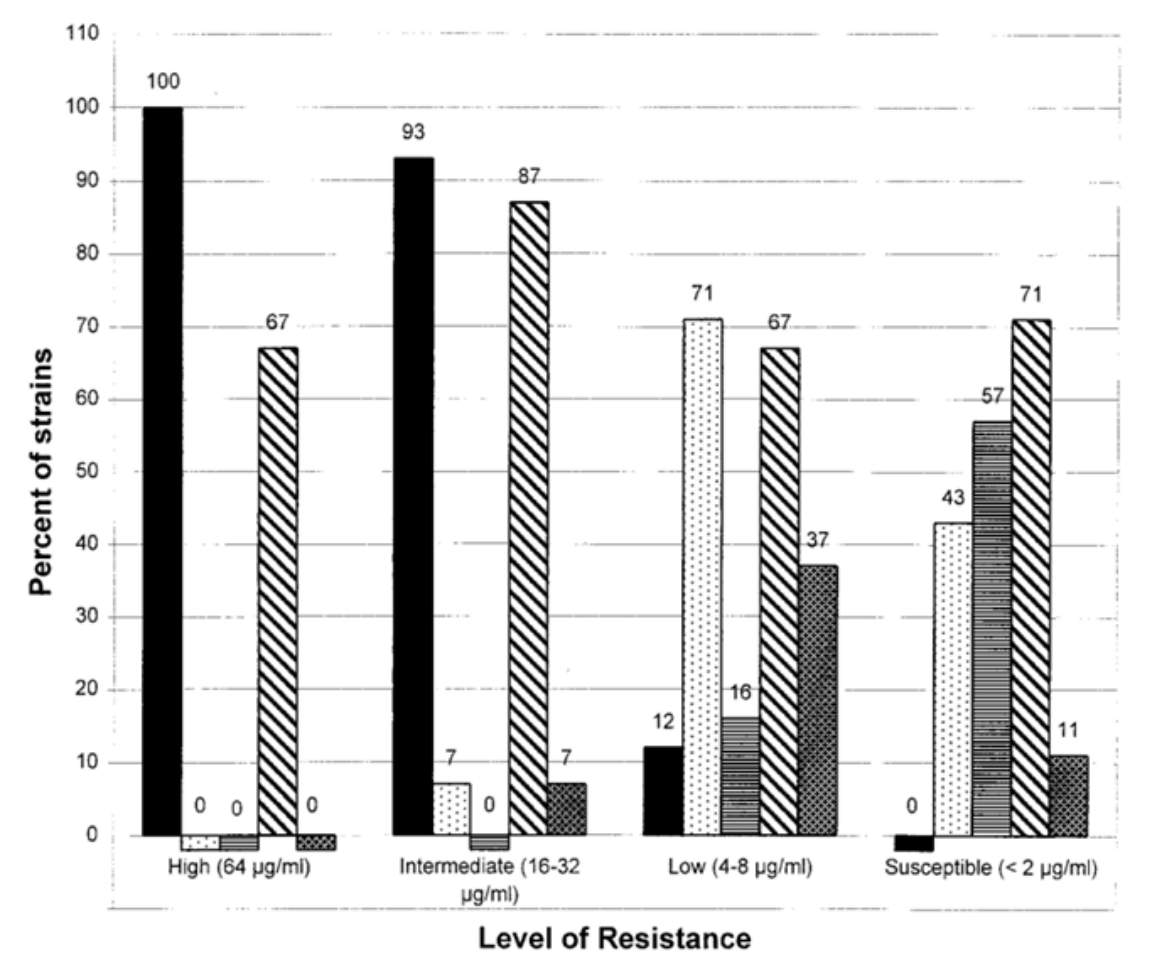
  Frequency: 1 in 10^6 to 10^9 cells

- Decreased intracellular accumulation

  Absence of porins

  Mutations within regulatory genes of active pumps results in increased expression of pumps

CLINICAL FQ RESISTANCE



parC* and *gyrA	**Solid bars**
parC	**Dotted bars**
No mutations	**Horizontal lines**
parE	**Diagonal lines**
Efflux	**Cross-hatched bars**

Antimicrob Agents Chemother. 44:3049-3054; 2000

LEVOFLOXACIN vs. *S. pneumoniae*

Antimicrobial Susceptibility Breakpoints and First-Step *parC* Mutations in *Streptococcus pneumoniae*: Redefining Fluoroquinolone Resistance

Sue Lim,*† Darrin Bast,*† Allison McGeer,*† Joyce de Azavedo,*† and Donald E. Low*†

Emerg Infect Dis. 9:833-837; 2003

METHODS

- Clinical MIC breakpoints (CLSI)

Levofloxacin:	≤ 2	susceptible
	4	intermediate
	≥ 8	resistant

- Micro/molecular MIC breakpoints

Sequenced *parC*, *gyrA*

Emerg Infect Dis. 9:833-837; 2003

ROLE OF ParC AND GyrA

Table 2. Number of isolates with ParC and GyrA amino acid substitutions and their corresponding levofloxacin MICs

MIC (µg/mL)	No. strains with amino acid substitutions in ParC (%)	ParC and GyrA (%)
2	48/82 (59)	0/29[a] (0)
4	5/8 (63)	3/8 (38)
8	0/10 (0)	10/10 (100)
≥16	0/15 (0)	15/15 (100)

[a]29/82 isolates were randomly examined for GyrA mutations.

Emerg Infect Dis. 9:833-837; 2003

WHY CAN THIS BE IMPORTANT?

TABLE 1. MICROBIOLOGIC CHARACTERISTICS OF *STREPTOCOCCUS PNEUMONIAE* ISOLATED BEFORE, DURING, OR AFTER THERAPY WITH ORAL LEVOFLOXACIN FROM FOUR PATIENTS WITH COMMUNITY-ACQUIRED PNEUMONIA.*

PATIENT NO.	SOURCE AND TIME OF CULTURE	SEROTYPE	PFGE PATTERN†	SUSCEPTIBILITY TO LEVOFLOXACIN‡	MINIMAL INHIBITORY CONCENTRATION§			AMINO ACID SUBSTITUTION	
					LEVOFLOXACIN	MOXIFLOXACIN	GATIFLOXACIN	IN PARC	IN GYRA
					μg/ml				
1	Sputum, before treatment	23F	A	S	1 (S)	0.12 (S)	0.25 (S)	—	—
	Sputum, after treatment	23F	A	R	8 (R)	1 (S)	2 (I)	S79F	S81F
2	Sputum, before treatment	6A	B	S	4 (I)	0.25 (S)	0.5 (S)	S79F	—
	Sputum, during treatment	6A	B	R	16 (R)	4 (R)	4 (R)	S79F	S81F
3	Blood, before treatment	14	C	R	16 (R)	4 (R)	2 (I)	S79F	S81Y
	Pleural fluid, during treatment	14	C	R	16 (R)	4 (R)	2 (I)	S79F and D83Y	S81Y
4	Sputum, during treatment	ND	ND	R	16 (R)	4 (R)	8 (R)	S79Y	E85K

N Engl J Med. 346:747-750; 2002

MACROLIDE CLASS

Parameter	Description
Mechanism of action	Bind reversibly to 50S ribosomal subunits, blocking the translocation reaction of polypeptide chain elongation
Activity rendered	Static
Route of administration	PO or IV
Distribution	Well, especially tissue and intracellular; no CNS
Half-life	1.5-41 hours; azithromycin 2-4 days in tissue
Excretion	Renal and biliary
Adverse effects	Nausea, vomit, diarrhea, hypersensitivity; reversible hearing loss with high dose + renal insufficiency

MACROLIDE RESISTANCE

- Size matters
- Methylation of ribosome
  - *ermA* → erythromycin ribosomal methylase
  - Macrolides can induce lincosamide, streptogramin resistance
- Expression of efflux pumps
  - Resistance to macrolides, not clindamycin

ERYTHROMYCIN RESISTANCE

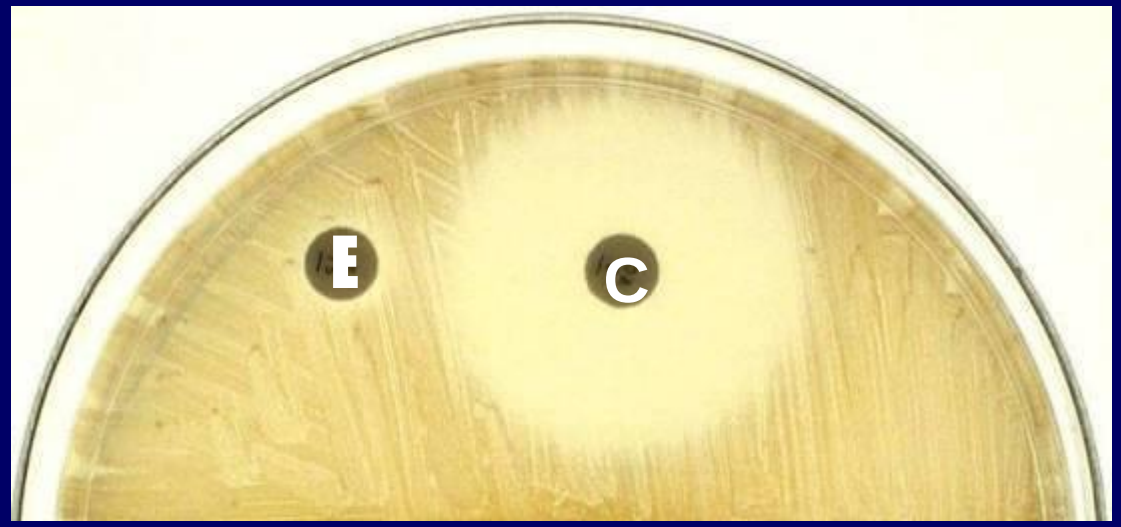
- Staphylococci and streptococci
- *msrA* → constitutive macrolide resistance
- *erm* gene cassette → inducible resistance

a.k.a. MLS_B locus

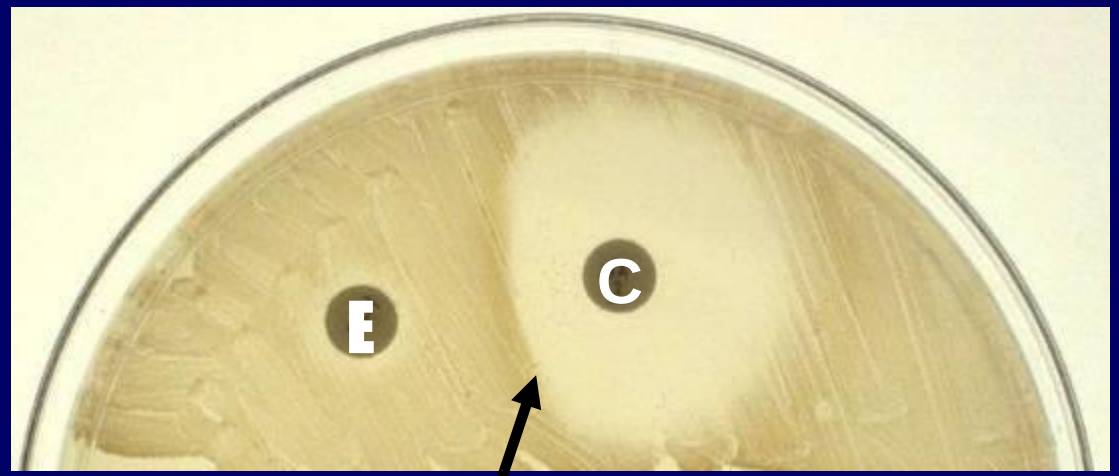
MACROLIDE
LINCOSAMIDE
STREPTOGRAMIN

ERYTHROMYCIN/CLINDAMYCIN TESTING

msrA-mediated erythromycin resistance

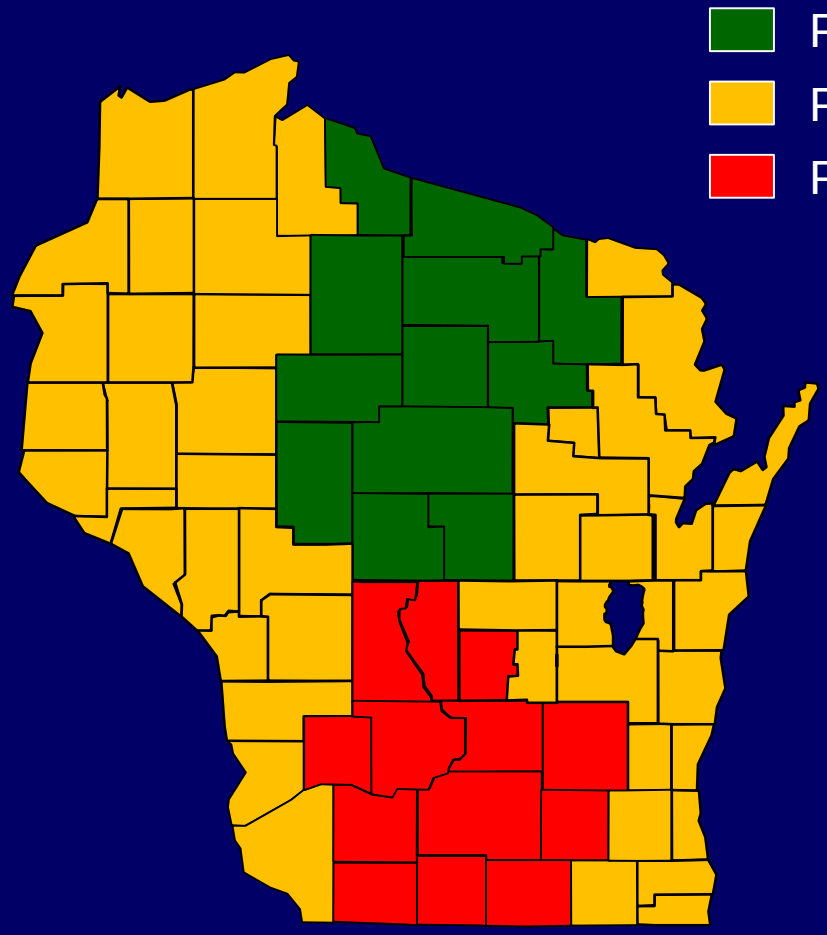


erm-mediated erythromycin resistance



Inducible clindamycin resistance

Staphylococcus aureus SURVEILLANCE



clindamycin
state mean 74.8%

- Percentage susceptible 5% or more greater than state mean
- Percentage susceptible ±5% of state mean
- Percentage susceptible 5% or more less than state mean

n = 310 Wisconsin isolates

Surveillance of Wisconsin Organisms for Trends in Antimicrobial Resistance and Epidemiology (SWOTARE)

48.4% erythromycin susceptibility statewide

86.8% clindamycin susceptibility statewide

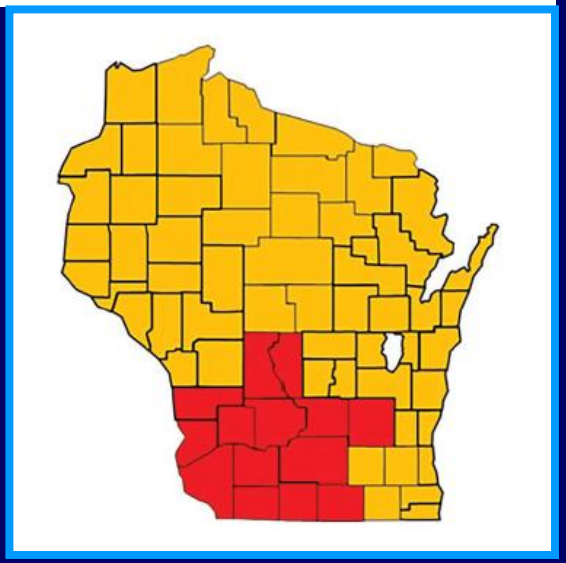
31.4% inducible clindamycin resistance (in 118 “D”-test eligible isolates)

74.8% clindamycin susceptibility statewide

S. pneumoniae SURVEILLANCE

Table 4: Comparison of antimicrobial susceptibility profiles for *Streptococcus pneumoniae* invasive isolates, Wisconsin 2006-2010 and 2016-2020

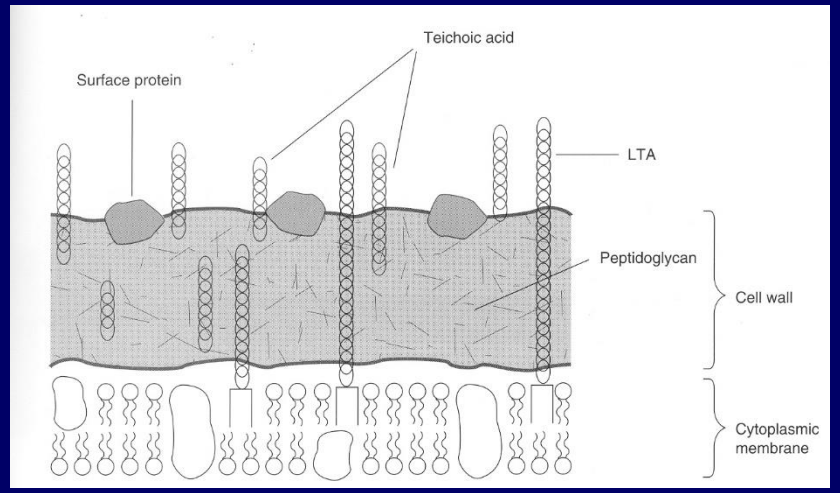
	Wisconsin, 2006-2010		Wisconsin, 2016-2020	
Antimicrobial Agent	n	Percentage Susceptible	n	Percentage Susceptible
Penicillin oral/CSF	1231	76.4[a]	1070	78.7
Penicillin non-CSF	1198	93.2[a]	1020	99.4[b]
Ceftriaxone CSF	1604	91.5[c]	1070	93.0
Ceftriaxone non-CSF	1612	96.2[c]	1070	99.1[b]
Erythromycin	1978	80.4	1070	64.8[b]



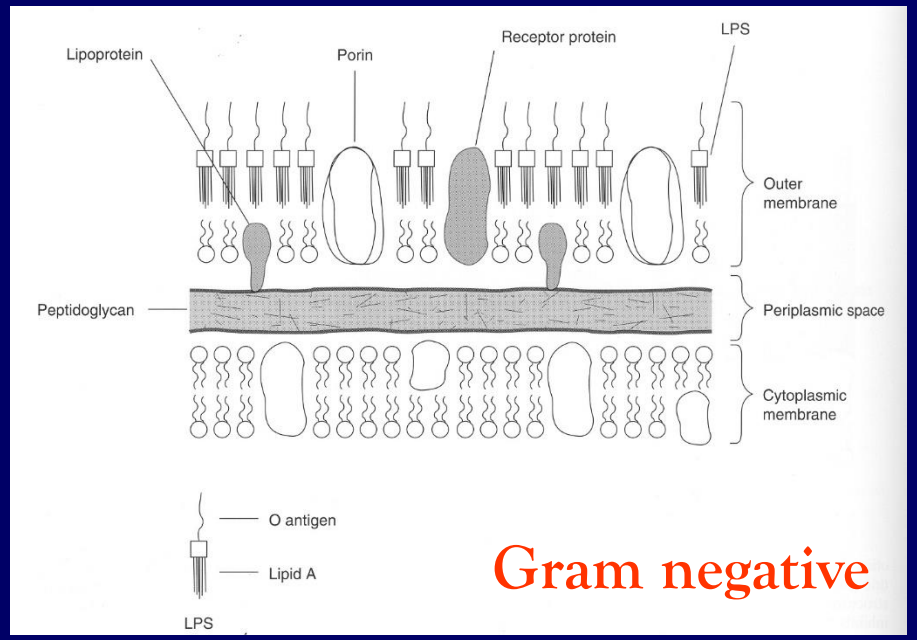
Clin Med Res. 20:185-194; 2022

GLYCOPEPTIDE RESISTANCE (INTRINSIC)

- Large size limits ability to penetrate Gram-negatives



Gram positive



GLYCOPEPTIDE RESISTANCE (ACQUIRED)

- Altered precursor formation

Peptidoglycan precursor, exiting from cytoplasmic membrane, terminates in alanine~alanine

Resistance genes promote change to alanine~lactate

1000-fold reduced affinity for vancomycin

vanA transposon (plasmid)
vanB transposon (plasmid)

vanC chromosomal
vanD chromosomal
vanE chromosomal
vanG chromosomal

Antimicrobial Stewardship: The Why, What, Who and How of Stewardship and the Lab's Integral Role

Alexander J. Lepak, MD, FIDSA
Associate Professor of Medicine
Medical Director, Antimicrobial Stewardship, UW Health
Chair, Antimicrobial Use Committee, UW Health
Division of Infectious Diseases
Department of Medicine
Univ of Wisconsin School of Medicine and Public Health
ajlepak@medicine.wisc.edu

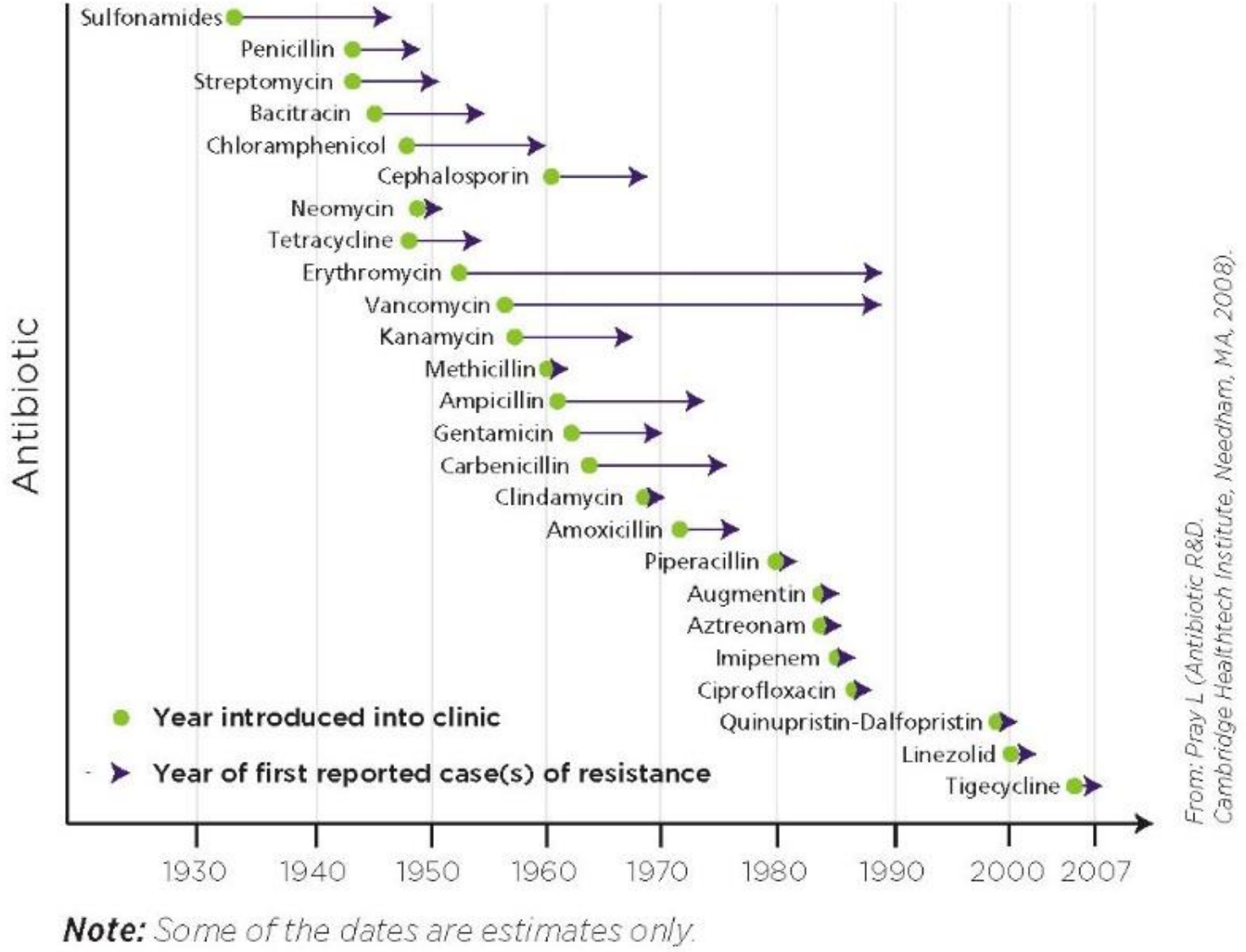
Disclosures

- Principle Investigator for an Investigator Initiated Research Grant funded by bioMérieux
- Past/Present advisor/content expert for USCAST, CLSI, FDA, GARDP (Europe), NIH/NIAID, and CMS (Regulation and Policy for Infectious Disease Stewardship Network in association with Rubrum Advising, Federation of American Hospitals, and Association of American Medical Colleges)
- Co-PI on numerous PK/PD drug development programs including setting optimal clinical breakpoints for approved and pre-clinical candidate therapies

Why Stewardship? – The Unique Dilemma of Antimicrobial use

- The #1 driver of antimicrobial resistance is use
- What you do (use) for one patient affects other current and future patients
  - There are societal repercussions to use, and as such antimicrobials should be viewed similarly to any other "shared natural resources", which often require complex cooperation for sustainability.
- Antibiotics are the only medication that use in one patient can significantly affect the efficacy of that drug for another patient
  - Antibiotics become less useful after market introduction

What kind of 'Tread-Life' do we get before Resistance



*And the Pipeline is relatively dry (whole separate topic)

The What – What is Stewardship?

- Conservation of resources (sustainability)
- Ensuring the optimal use of finite resources
- Fair and equitable application of stewardship
- Consideration of the current situation and future needs
- Consideration of an individual’s and societal needs
- Adaptive management

Antimicrobial Stewardship at UW

- ***Daily monitoring and review of all inpatient antimicrobial use***
- ***Drug consultation and restricted drug approvals***
- ***Collaborative & interdisciplinary teamwork***
- ***Tracking and reporting of antimicrobial use and resistance data***
- ***Commitment to education and quality improvement***
- ***Fulfillment of CDC 7 core elements of hospital stewardship***

https://www.cdc.gov/antibiotic-use/hcp/core-elements/index.html
https://www.idsociety.org/practice-guideline/implementing-an-ASP/
https://iris.who.int/bitstream/handle/10665/340709/9789289054980-eng.pdf

The Who and the How – Teamwork makes the Dream work

- The Stewardship team is made up of 7 core physicians, 4 core pharmacists, 2 PGY2 Pharmacist trainees
- Program processes are performed 7 days with coverage from 7am-10pm

Alex Lepak, MD, FIDSA;
Medical Director UWHealth Antimicrobial Stewardship;
Chair AMUS Committee;
Co-Chair WINSPIRE;
Co-Director UWHealth Ambulatory Stewardship

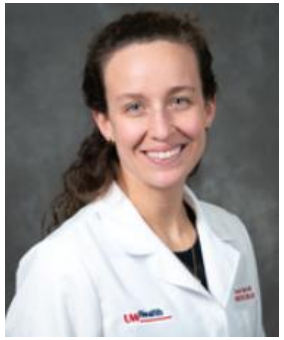
David Andes, MD, FIDSA;
Division Chief of Infectious Diseases

Brittany Lehrer, MD, MPH;
Medical Director Pediatric Antimicrobial Stewardship

Joseph McBride, MD;
Medical Director of Antimicrobial Stewardship at Meriter Hospital

Swapnil Lanjewar, MD;
Medical Director of Antimicrobial Stewardship at Select Hospital

Lindsay Taylor, MD;
Antimicrobial Stewardship Coordinator, State of Wisconsin



Jessica Tischendorf, MD;
Program Director for ID Fellowship

Brian Buss, PharmD;
Director of ID Pharmacy

Courtney Baus, PharmD;
Co-Director UWHealth Ambulatory Stewardship

Megan Wimmer, PharmD;
Director of ID Pharmacy PGY-2

Jill Strayer, PharmD;
Director of Pediatric ID Pharmacy and Ambulatory ID Clinic Pharmacy

The How – How do we do Stewardship?

- We will discuss the main methods UW has decided to prioritize for antimicrobial stewardship, but by no means is there a single “right way”, method, etc.

Guidelines, Delegation Protocols, Order sets, etc.

- AMS service provides input on, drafts, and champions in total 92 order sets, guidelines, and protocols within the UWHealth system
  - Includes inpatient and ambulatory care
- There are ~150 pre-op/operative/procedural order sets (have to review and implement prophylaxis when indicated)
- Numerous Pharmacy dosing delegations and guidelines
  - E.g. Vanco, Dapto, Beta-lactams, etc.

Leveraging PK/PD to treat GNR – Beta-lactam Prolonged Infusion Protocols

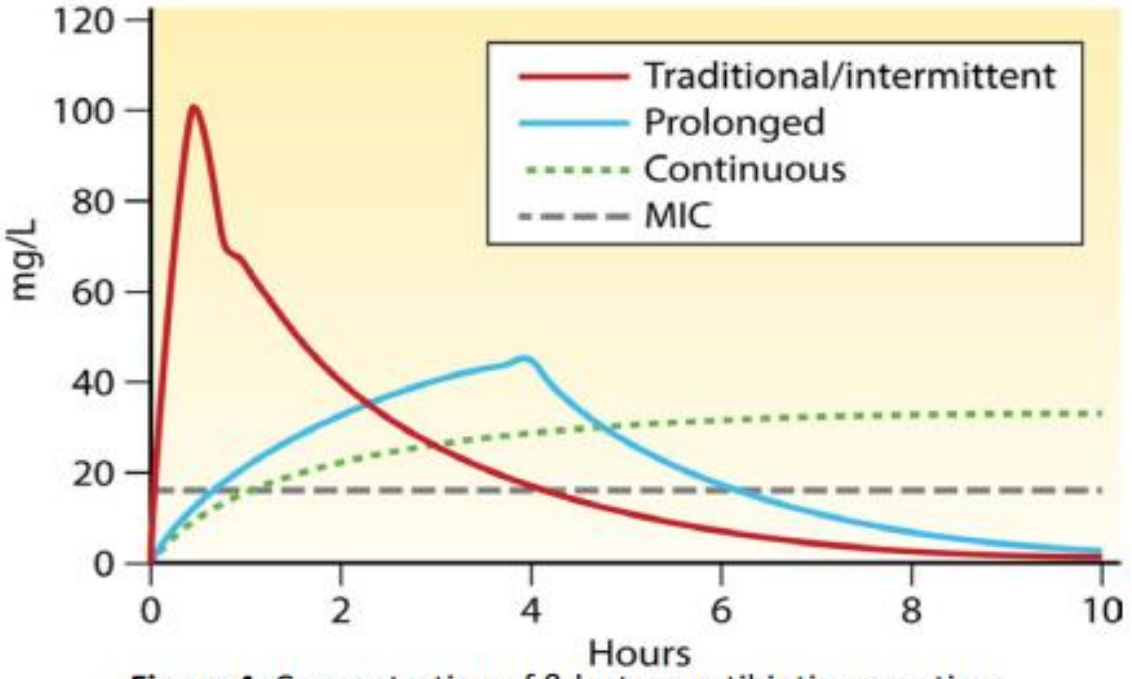


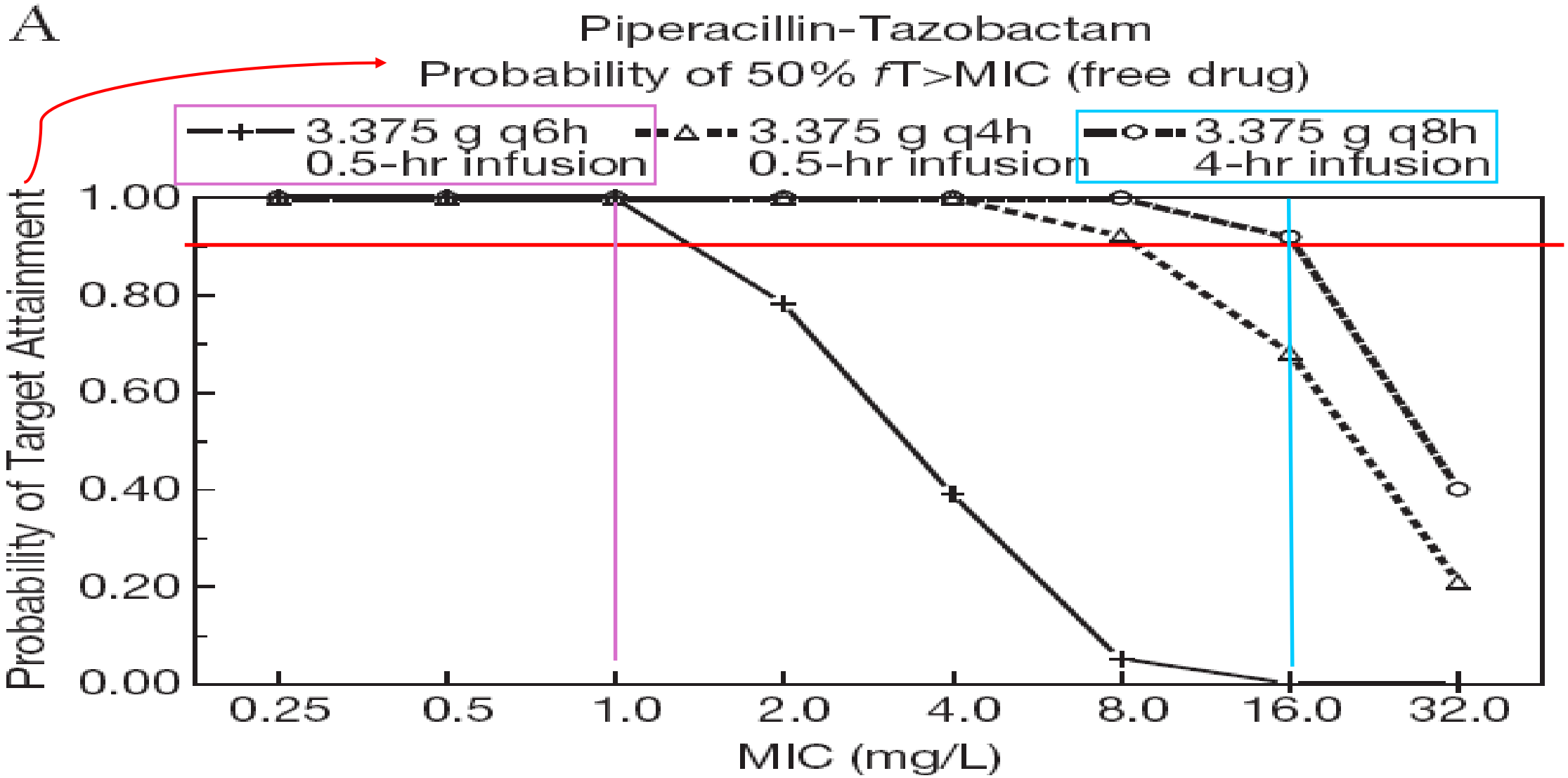
Figure 4. Concentration of β-lactam antibiotics over time
http://cmr.asm.org/content/29/4/759/F4.large.jpg

Standard = 30 min
Prolonged = 3 or 4 hr "slow" infusion
Continuous = slow drip over ~23h

Table 1. PK/PD optimized dosing regimens

Drug	Est CrCL (mL/min)	Empiric			Definitive Therapy	
		Sepsis, Septic Shock Indication	Non-sepsis[A] Indication	Obese[B]	Non-obese	Obese[B]
Cefepime[C] - 4hr infusion	> 50	2 g IV Q8H	1 g IV Q6H	Based on indication	1 g IV Q6H if MIC ≤4 or no organism is cultured	
	30 – 50	2 g IV Q12H	1 g IV Q8H		1 g IV Q8H if MIC ≤4 or no organism is cultured	
	15 – 29	2 g IV Q24H	1 g IV Q12H		1 g IV Q12H if MIC ≤4 or no organism is cultured	
	<15 / HD	1 g IV Q24H	1 g IV Q24H		1 g IV Q24H if MIC ≤4 or no organism is cultured	
Piperacillin/ tazobactam - 4hr infusion	> 20	4.5 g IV Q8H	3.375 g IV Q8H	4.5 g IV Q8H	3.375 g IV Q8H	4.5 g IV Q8H
	< 20	4.5 g IV Q12H	3.375 g IV Q12H	4.5 g IV Q12H	3.375 g IV Q12H	4.5 g IV Q12H
Meropenem[C] - 3hr infusion	> 50	500 mg IV Q6H	500 mg IV Q8H	500 mg IV Q6H	500 mg IV Q8H if MIC ≤2 or no organism is cultured	
	26 – 50	500 mg IV Q8H	500 mg IV Q8H	500mg IV Q8H	500 mg IV Q8H if MIC ≤2 or no organism is cultured	
	10 – 25	500 mg IV Q12H	500 mg IV Q12H	500mg IV Q12H	500 mg IV Q12H if MIC ≤2 or no organism is cultured	
	< 10 / HD	500 mg IV Q24H	500 mg IV Q24H	500mg IV Q24H	500 mg IV Q24H if MIC ≤2 or no organism is cultured	

PK/PD target attainment – Piperacillin/Tazobactam



Lodise TP, Lomaestro BP, Drusano GL. Application of antimicrobial pharmacodynamic concepts in clinical practice: focus on beta-lactams. Pharmacotherapy 2006;26:1320-32

Audit and Feedback

- Prospective audit and feedback
  - Every patient on an antibiotic (more than 1x prophylaxis) is reviewed during their stay, and may be reviewed more than once
  - >300 patients reviewed each day
  - ~20 recommendations to optimize therapy each day
  - 93% acceptance rate for AMS recommendations
- Majority of interventions are for
  - De-escalation/discontinue
  - Limit/set a duration
  - Remove unnecessary duplicative therapy
  - IV to oral
  - Optimize
    - Switch drug
    - Dose optimization

Cascade Reporting

- This is a good way to "nudge" clinicians to use preferred, first-line agents and reserve agents of last resort for MDRO
  - "Out of sight, out of mind"
- First-line drugs are viewable by everyone, those drugs for only resistant organisms or "nuanced" situations remain hidden
  - The hidden results get auto-released if resistance is present
  - Providers can call to obtain hidden results if they have specific clinical scenarios that require them
- Use a multi-d group to discuss, discuss, discuss

CLSI M100-ED35:2025 Performance Standards for Antimicrobial Susceptibility Testing, 35th Edition

Search within this Document

< Previous | Next >

Table 1A-1. Enterobacterales (excluding *Salmonella* and *Shigella* spp.)[a]

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Ampicillin			
Cefazolin	Cefuroxime		
Cefotaxime or ceftriaxone[b]	Cefepime[c]		
	Ertapenem Imipenem Meropenem	Cefiderocol	
		Ceftazidime-avibactam	
		Imipenem-relebactam	
		Meropenem-vaborbactam	
Amoxicillin-clavulanate Ampicillin-sulbactam			
Piperacillin-tazobactam			
Gentamicin	Tobramycin	Plazomicin	
	Amikacin		
Ciprofloxacin Levofloxacin			
Trimethoprim-sulfamethoxazole			
	Cefotetan Cefoxitin		
	Tetracycline		
			Aztreonam[d]
			Ceftaroline[b]
			Ceftazidime[b]
			Ceftolozane-tazobactam

Cascade Reporting

- Depends much on your formulary, patient population, antibiogram, and resources
  - Requires IS build
  - Requires a process to be able to release hidden results with appropriate clinical request
  - Requires a thoughtful process for what to do about hidden results that are “resistant”
- Works best in ambulatory environment to “nudge” providers to optimized first-line, second-line, etc. drugs for common conditions
  - E.g. UTI

Restricted Formulary

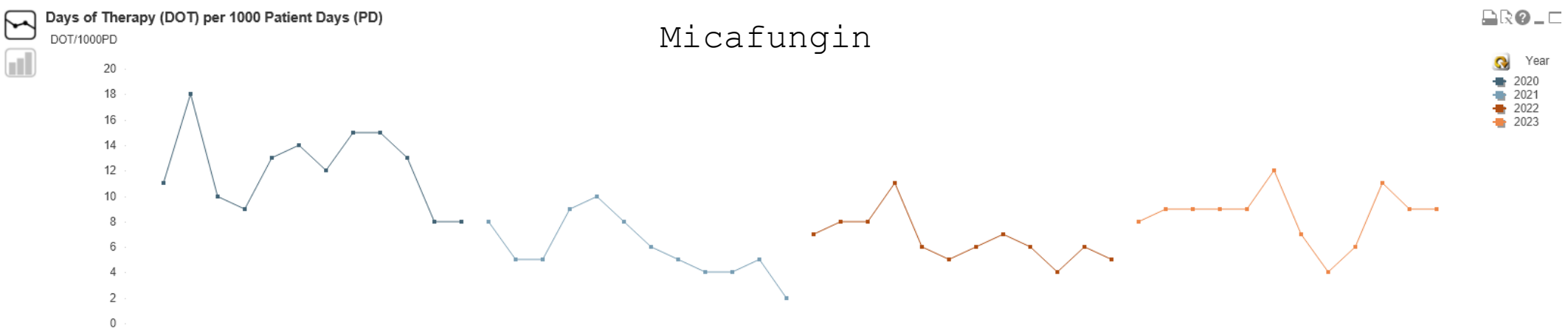
- If resources exist, an alternative to cascade reporting is having drug restrictions
  - Prior-approval needed on select antimicrobials
  - Requires infectious disease expertise
  - Requires resources to staff the approval process
  - Requires institutional “buy-in” and support from the highest levels
- A restricted formulary (i.e. prior approval) may obviate the importance/significance of cascade reporting
- We have found the most juice from the squeeze occurs with restricted formulary for inpatients and cascade reporting for ambulatory patients

Prior Authorization/Restricted Formulary

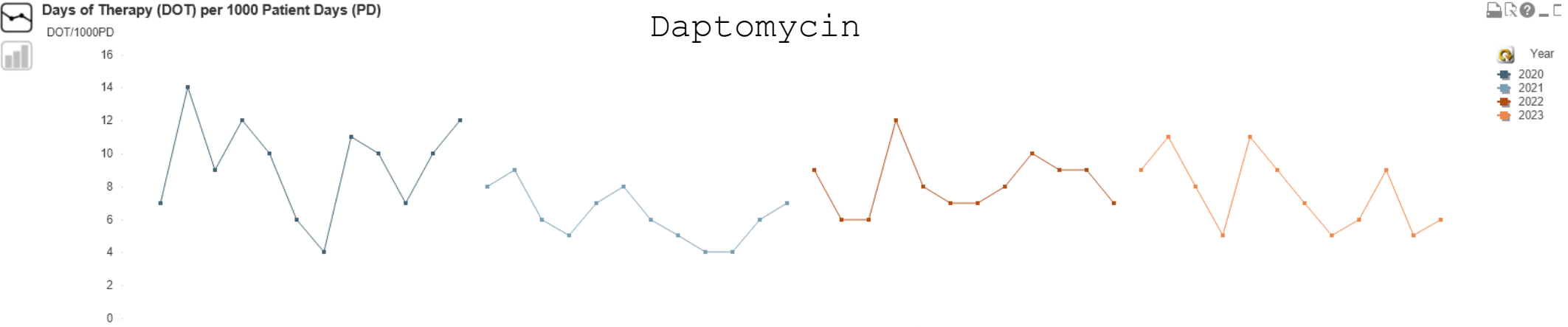
- 58 restricted antimicrobials
- Stewardship services (mostly physician covering stewardship) get on average >30 restricted drug requests weekly
  - Why do we manage so many restricted drugs?
    - High risk/reward drugs
    - Drugs used for critical infectious disease syndromes
    - Drugs of last resort for AMR
    - Responsible resource utilization
- Restricted drug pager is often an opportunity to educate on optimal drug use and collaborate to improve patient outcomes

Examples of restricted drug use trends

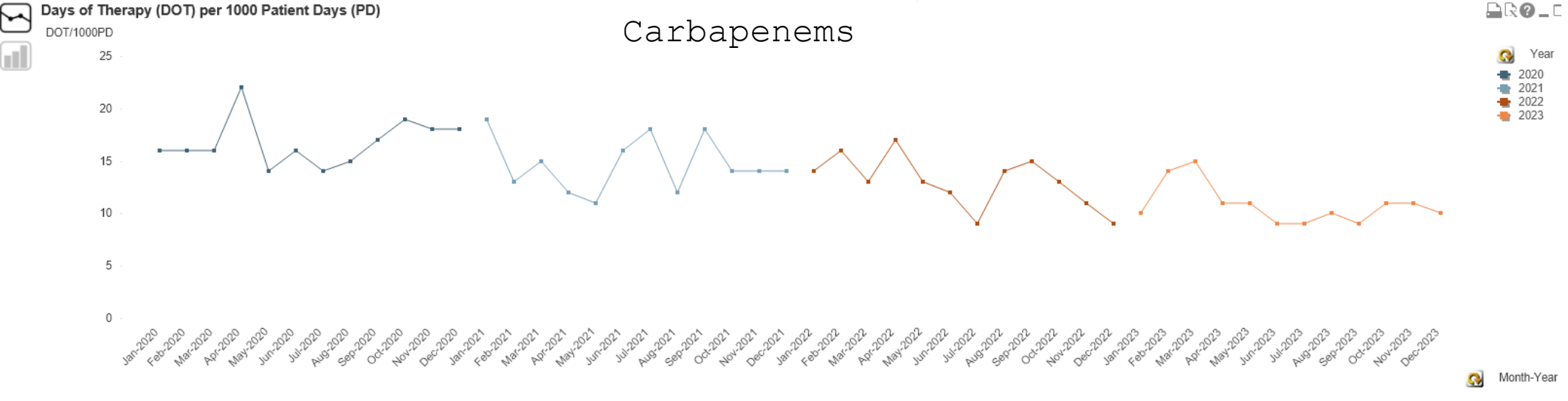
Micafungin



Daptomycin



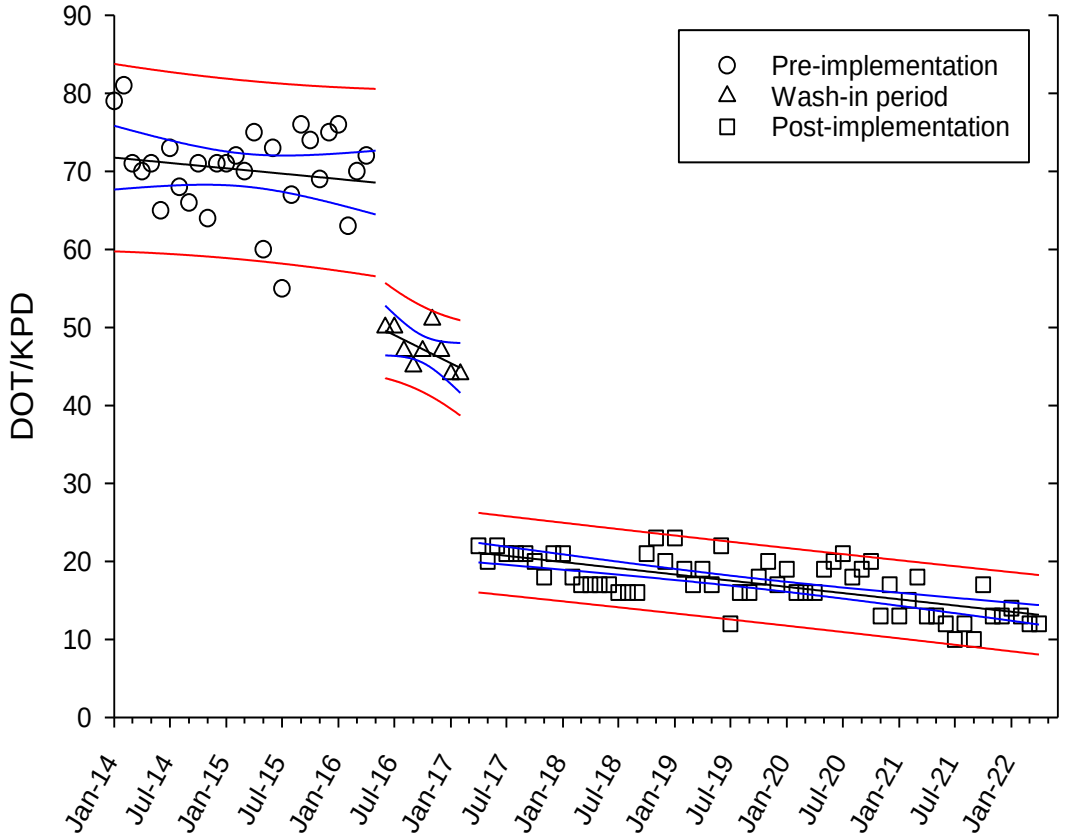
Carbapenems

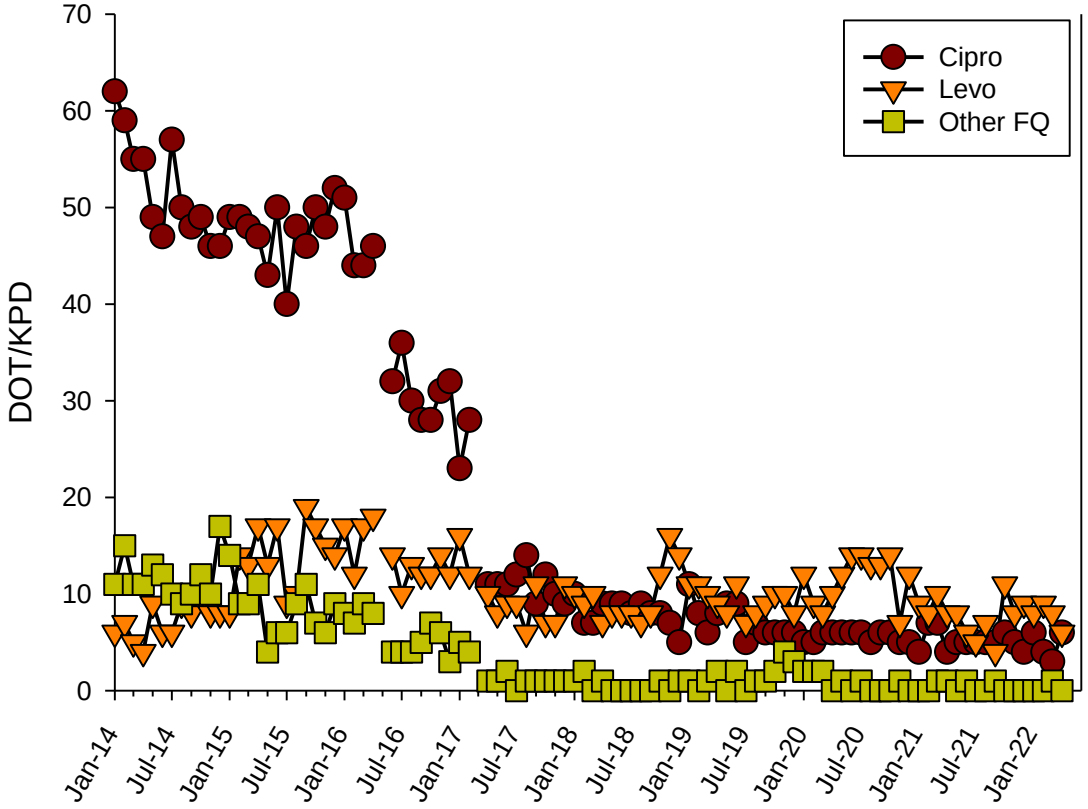


Fluoroquinolone Restriction – A success story

- 6 FDA/black box warnings from 2008-2018
- One of the highest risk antibiotics for c diff
  - Also published evidence that **use on the ward increases c diff risk for the whole ward** – collateral damage not to just index patient
- One of the most over-prescribed antibiotics with rapidly increasing resistance in community and hospital
- Do not have almost any infectious disease syndrome where they are a first-line option without alternatives
- Their benefit is outpatient>>>inpatient

Inpatient Fluoroquinolone Restriction and Use trends

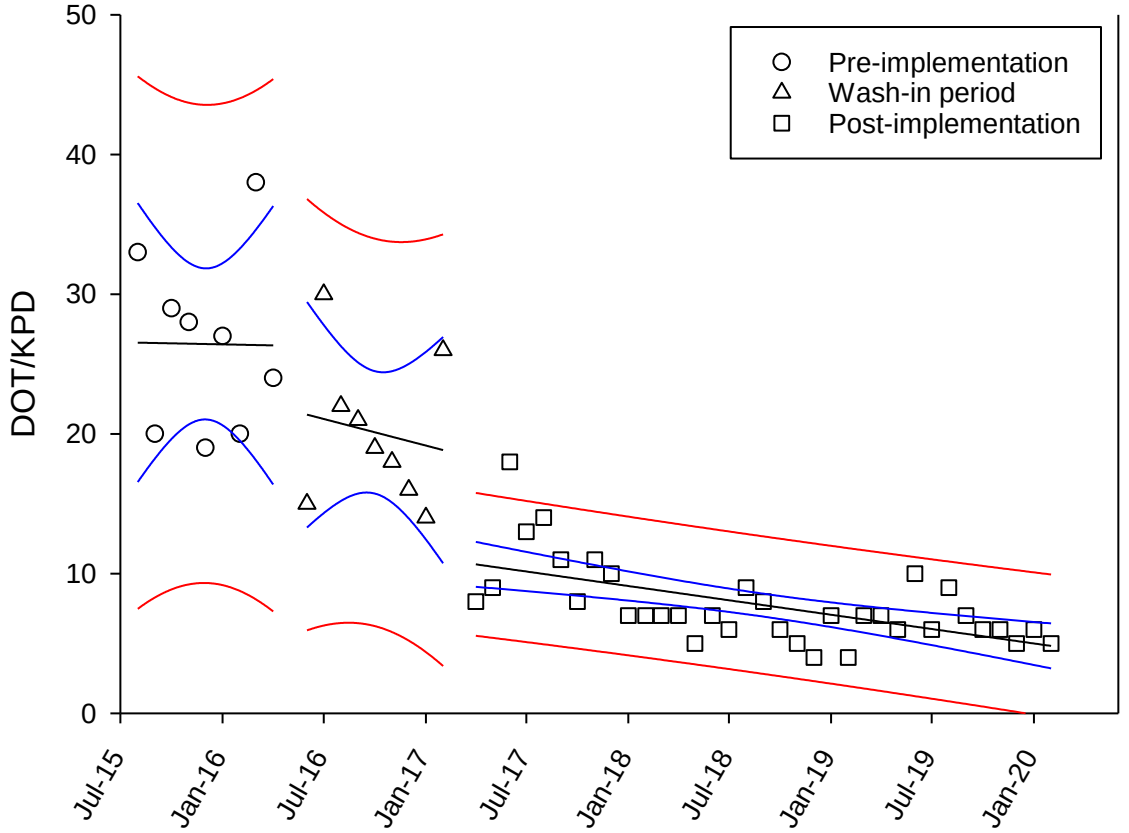




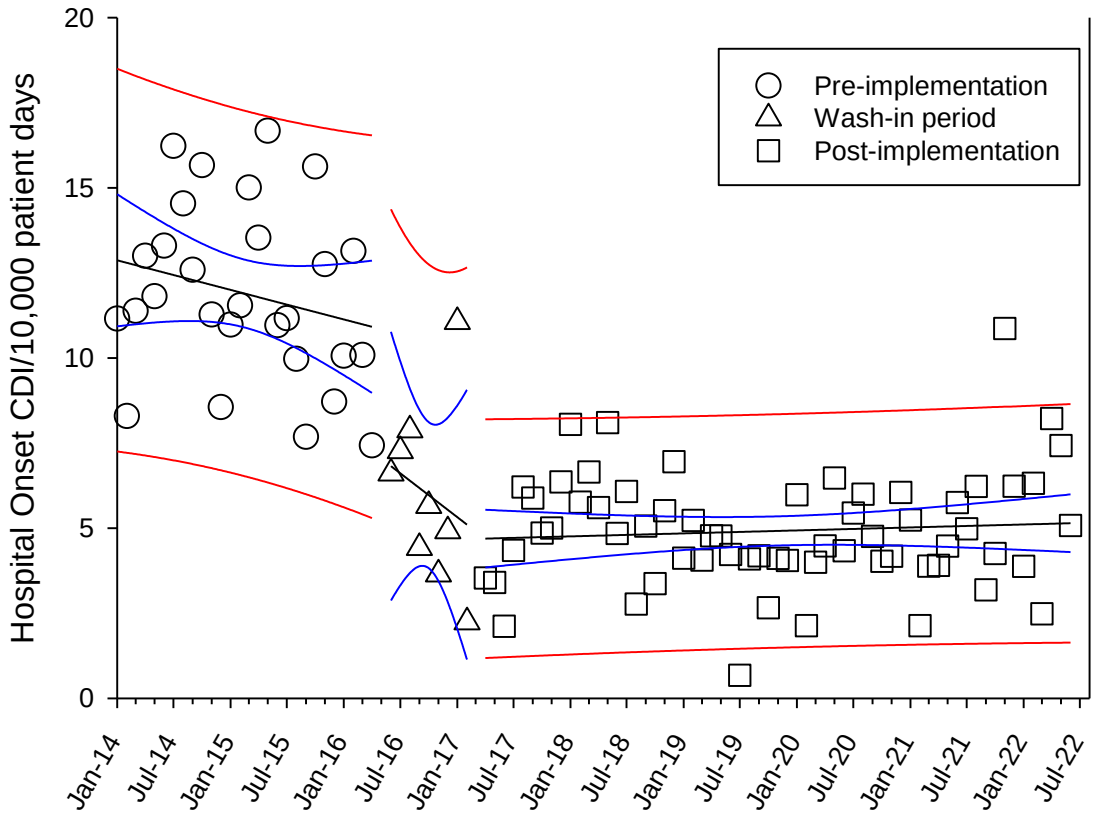
Pre-implementation = no Fq restriction in place
Wash-in = restriction in ICU and Heme/Onc wards only
Post-implementation = hospital wide restriction

Fluoroquinolone Restriction – Associated effects

FQ Use at Unrestricted Hospital



Hospital Onset CDI



Using the Lab for Stewardship – The Laboratory 'Nudge' – Several Examples

- **No Staph/No Pseudomonas**

CULTURE, RESPIRATORY W/WO GRAM STAIN (UWH)	Moderate Endogenous Flora Negative for S. aureus/MRSA and P. aeruginosa.
GRAM STAIN - CULTURE, RESPIRATORY (UWH)	<10/LPF Squamous Epithelial Cells <10/LPF Neutrophils <10/LPF Mononuclear Cells Rare Gram-Positive Cocci, Pairs >10/LPF Respiratory Epithelial Cells

Resulting Agency: MAIN

CULTURE, RESPIRATORY W/WO GRAM STAIN (UWH)	Moderate to many Corynebacterium sp. ! No further workup. Test methodology for identification is mass spectrometry. Few Endogenous Flora Negative for S. aureus/MRSA and P. aeruginosa. Mass Spectrometry ID: The performance characteristics of this test were validated by UWHC Clinical Laboratories. The US Food and Drug Administration (FDA) has not approved or cleared this test; however, FDA approval or clearance is currently not required for clinical use of this test. The results are not intended to be used as the sole means for clinical diagnosis or patient management decisions. The UWHC Clinical Laboratories is authorized under Clinical Laboratory Improvement Amendments (CLIA) to perform high-complexity testing.

CULTURE, RESPIRATORY W/WO GRAM STAIN (UWH)	Negative for S. aureus/MRSA and P. aeruginosa. The negative predictive value of a Gram stain with no bacteria is 95%. Specimen has a predominance of WBCs and will be screened for the presence of Staphylococcus aureus/MRSA and Pseudomonas aeruginosa.
GRAM STAIN - CULTURE, RESPIRATORY (UWH)	<10/LPF Squamous Epithelial Cells 10-25/LPF Neutrophils No organisms seen.

Resulting Agency: MAIN

CULTURE, RESPIRATORY W/WO GRAM STAIN (UWH)	Few Candida albicans ! Considered part of endogenous flora. Moderate Endogenous Flora Negative for S. aureus/MRSA and P. aeruginosa.
GRAM STAIN - CULTURE, RESPIRATORY (UWH)	No squamous epithelial cells seen. >25/LPF Neutrophils Few Pleomorphic Gram-Positive Rods Few Gram-Positive Cocci, Pairs

Resulting Agency: MAIN

No Staph/No Pseudomonas

- After this behavioral nudge was implemented, prescribers were 34% ($p<0.01$) and 5.5-fold more likely to de-escalate antibiotics than when the report only stated "commensal respiratory flora".

	Early Period (2016-17)	Late period (2018-19)
Empiric MRSA/PSA coverage	100%	90%
De-escalation of MRSA active agents when "no staph/no pseudomonas" reported	71.4%	84.3%
De-escalation of PSA active agents when "no staph/no pseudomonas" reported	70.5%	75.8%

Lab "Comments"

Use your comments section and reporting wisely

- Stewardship and Lab meet almost monthly to discuss reporting comments

CULTURE, BLOOD, BACTERIA AND YEAST (UWH)

Methicillin-RESISTANT Staphylococcus aureus !!

METHICILLIN RESISTANT STAPH. AUREUS; PATIENT REQUIRES ISOLATION

Aerobic Bottle — Hours Until Positive: 22.4

MRSA PCR is positive.
S. aureus PCR is positive.

Resulting Agency: MAIN

Susceptibility

Methicillin-RESISTANT Staphylococcus aureus (1)

Antibiotic	Interpretation	MIC	Method	Status
Clindamycin (UWHC)	Resistant		MIC (UG/ML)	Final
Daptomycin (UWHC)	Susceptible	0.5	MIC (UG/ML)	Final
Doxycycline (UWHC)	Susceptible Doxycycline should not be used alone for serious infections.	<=0.5	MIC (UG/ML)	Final
Gentamicin (UWHC) *	Susceptible Gentamicin should not be used alone for therapy.	<=0.5	MIC (UG/ML)	Final
Linezolid (UWHC)	Susceptible	2	MIC (UG/ML)	Final
Moxifloxacin (UWHC) *	Resistant	4	MIC (UG/ML)	Final
Oxacillin;Diclox	Resistant Susceptibility to oxacillin predicts susceptibility to cephalexin, cefuroxime, and cefazolin.	>=4	MIC (UG/ML)	Final
Rifampin (UWHC) *	Susceptible Rifampin should not be used alone for therapy.	<=0.5	MIC (UG/ML)	Final
Tetracycline (UWHC)	Susceptible Tetracycline should not be used alone for serious infections.	<=1	MIC (UG/ML)	Final
Tigecycline (UWHC)	Susceptible	<=0.12	MIC (UG/ML)	Final
Sulfa & Trimeth (UWHC)	Susceptible Sulfa & Trimeth should not be used alone for serious infections.	<=10	MIC (UG/ML)	Final
Vancomycin (UWHC)	Susceptible	1	MIC (UG/ML)	Final

* Suppressed Antibiotic

MDRO/ESBL/AmpC comments

CULTURE, URINE W/WO GRAM STAIN (UWH)

>100,000 CFU/mL Escherichia coli !

MULTIDRUG-RESISTANT ORGANISM; PATIENT REQUIRES ISOLATION
Isolate possesses extended spectrum beta-lactamase (ESBL) activity.

Resulting Agency: MAIN

Susceptibility

Escherichia coli (1)

Antibiotic	Interpretation	MIC	Method	Status
Amox Clavulanate (UWHC)	Susceptible	8	MIC (UG/ML)	Final
Ampicillin (UWHC)	Resistant	>=32	MIC (UG/ML)	Final
Aztreonam (UWHC) *	Susceptible	<=1	MIC (UG/ML)	Final
Cefazolin (UWHC)	Resistant	>=32	MIC (UG/ML)	Final
Cefepime (UWHC)	Susceptible	2	MIC (UG/ML)	Final
Cefotaxime (UWHC)	Resistant	>=64	MIC (UG/ML)	Final
	Cefotaxime use is restricted to neonates or infants with hyperbilirubinemia.			
Cefoxitin (UWHC)	Susceptible	<=4	MIC (UG/ML)	Final
Cefpodoxime (UWHC)	Resistant	>=8	MIC (UG/ML)	Final
Ceftazidime (UWHC) *	Susceptible	<=0.5	MIC (UG/ML)	Final
Ceftazidime avibactam (UWHC) *	Susceptible	<=0.12	MIC (UG/ML)	Final
Ceftolozane tazobactam (UWHC) *	Susceptible	<=0.25	MIC (UG/ML)	Final
Ceftriaxone (UWHC)	Resistant	>=64	MIC (UG/ML)	Final
Cefuroxime (UWHC)	Resistant	>=64	MIC (UG/ML)	Final
Ciprofloxacin (UWHC)	Susceptible	<=0.06	MIC (UG/ML)	Final
Doxycycline (UWHC) *	Resistant	>=16	MIC (UG/ML)	Final
Ertapenem (UWHC) *	Susceptible	<=0.12	MIC (UG/ML)	Final
Gentamicin (UWHC)	Susceptible	<=1	MIC (UG/ML)	Final
Levofloxacin (UWHC)	Susceptible	<=0.12	MIC (UG/ML)	Final
Meropenem (UWHC)	Susceptible	<=0.25	MIC (UG/ML)	Final
Meropenem-vaborbactam (UWHC) *	Susceptible	<=0.5	MIC (UG/ML)	Final
	Breakpoints are based on an adult dosage regimen of 4 g (2g meropenem + 2g vaborbactam) every 8h administered over 3h.			
Moxifloxacin (UWHC) *	Susceptible	<=0.25	MIC (UG/ML)	Final
Nitrofurantoin (UWHC)	Susceptible	<=16	MIC (UG/ML)	Final
Pip Tazobactam (UWHC)	Susceptible	<=4	MIC (UG/ML)	Final
Tigecycline (UWHC) *	Susceptible	<=0.5	MIC (UG/ML)	Final
Tobramycin (UWHC)	Susceptible	<=1	MIC (UG/ML)	Final
Sulfa & Trimeth (UWHC)	Resistant	>=320	MIC (UG/ML)	Final
ESBL Confirm *	Positive	Positive	MIC (UG/ML)	Final
Fosfomycin (UWHC)	Resistant		KIRBY BAUER	Final

* Suppressed Antibiotic

CULTURE, BLOOD, BACTERIA AND YEAST (UWH)

Klebsiella (Enterobacter) aerogenes !!

Test methodology for identification is mass spectrometry.
Isolate is intrinsically resistant to ampicillin, amoxicillin-clavulanate, ampicillin-sulbactam, 1st generation cephalosporins, and cephamycins (e.g. cefoxitin, cefotetan). NOTE: In circumstances where the bio-burden of micro-organisms is estimated to be high and therapy is intended to exceed 4 days, this organism may develop resistance during therapy with amp/sulbactam and 3rd generation cephalosporins such as ceftriaxone and cepodoxime. When the bio-burden is estimated to be low, (i.e. after surgical debridement/washout), therapy with these antibiotics may be safely considered for approximately one week.

Aerobic Bottle	Hours Until Positive
	11.8

Anaerobic Bottle	Hours Until Positive
	13.0

Meningitis Reporting

Susceptibility

	Streptococcus pneumoniae MIC (UG/ML)	
Ceftriaxone (meningitis) (UWHC)	0.5	Susceptible
Ceftriaxone (nonmeningitis) (UWHC)	0.5	Susceptible
Meropenem (UWHC)	0.25	Susceptible
Penicillin (oral penicillin V) (UWHC)	0.5	Intermediate
Penicillin parenteral (meningitis) (UWHC)	0.5	Resistant
Penicillin parenteral (nonmeningitis) (UWHC)	0.5	Susceptible

Near Miss

CULTURE, BLOOD, BACTERIA AND YEAST

Status: Edited Result - FINAL

Test Result Released: Yes (not seen)

Specimen Information: Antecubital, Right; Blood

0 Result Notes

CULTURE, BLOOD, BACTERIA AND YEAST (UWH)

Escherichia coli !!

```
Test methodology for identification is mass spectrometry.
MULTIDRUG-RESISTANT ORGANISM; PATIENT REQUIRES ISOLATION
Isolate possesses extended spectrum beta-lactamase (ESBL) activity.
```

Aerobic Bottle	**Hours Until Positive**
	11.1
Anaerobic Bottle	**Hours Until Positive**
	10.9

```
Mass Spectrometry ID: The performance characteristics of this test wer
(FDA) has not approved or cleared this test; however, FDA approval or
are not intended to be used as the sole means for clinical diagnosis o
under Clinical Laboratory Improvement Amendments (CLIA) to perform hig
```

Resulting Agency: MAIN

Susceptibility

Escherichia coli (1)

Antibiotic	Interpretation	MIC	Method	Status
Amox Clavulanate (UWHC)	Susceptible	4	MIC (UG/ML)	Final
Ampicillin (UWHC)	Resistant	>=32	MIC (UG/ML)	Final
Aztreonam (UWHC) *	Susceptible	2	MIC (UG/ML)	Final
Cefazolin (UWHC)	Resistant	>=32	MIC (UG/ML)	Final
Cefepime (UWHC)	Susceptible	2	MIC (UG/ML)	Final
Cefotaxime (UWHC)	Resistant Cefotaxime use is restricted to neonates or infants with hyperbilirubinemia.	>=64	MIC (UG/ML)	Final
Cefoxitin (UWHC)	Susceptible	<=4	MIC (UG/ML)	Final
Cefpodoxime (UWHC)	Resistant	>=8	MIC (UG/ML)	Final
Ceftazidime (UWHC) *	Intermediate	8	MIC (UG/ML)	Final
Ceftazidime avibactam (UWHC) *	Susceptible	<=0.12	MIC (UG/ML)	Final
Ceftolozane tazobactam (UWHC) *	Susceptible	<=0.25	MIC (UG/ML)	Final
Ceftriaxone (UWHC)	Resistant	>=64	MIC (UG/ML)	Final

Stewardship Update to Culture Comment

CULTURE, BLOOD, BACTERIA AND YEAST (Acc# 25UH-074MI00226) (Order 690474975)

Status: Edited Result - FINAL (Collected: 3/15/2025 20:26)

Acc #: 25UH-074MI00226

CULTURE, BLOOD, BACTERIA AND YEAST Order: 690474975

Status: Edited Result - FINAL

Test Result Released: Yes (not seen)

Specimen Information: Antecubital, Right; Blood

0 Result Notes

CULTURE, BLOOD, BACTERIA AND YEAST (UWH)

Escherichia coli !!

Test methodology for identification is mass spectrometry.
MULTIDRUG-RESISTANT ORGANISM; PATIENT REQUIRES ISOLATION
Isolate possesses extended spectrum beta-lactamase (ESBL) activity.

	Hours Until Positive
Aerobic Bottle	11.1
Anaerobic Bottle	10.9

Mass Spectrometry ID: The performance characteristics of this test were validated by UWHC Clinical Laboratories. The US Food and Drug Administration (FDA) has not approved or cleared this test; however, FDA approval or clearance is currently not required for clinical use of this test. The results are not intended to be used as the sole means for clinical diagnosis or patient management decisions. The UWHC Clinical Laboratories is authorized under Clinical Laboratory Improvement Amendments (CLIA) to perform high-complexity testing.

Resulting Agency: MAIN

Susceptibility

Escherichia coli (1)

Antibiotic	Interpretation	MIC	Method	Status
Amox Clavulanate (UWHC)	Susceptible	4	MIC (UG/ML)	Final

Amoxicillin-clavulanate does not predict
ampicillin-sulbactam susceptibility.
Regular, oral amoxicillin-clavulanate
(Augmentin 875/125mg) should not be used for
blood stream or other serious infections.

Ampicillin (UWHC)	Resistant	>=32	MIC (UG/ML)	Final
Aztreonam (UWHC) *	Susceptible	2	MIC (UG/ML)	Final
Cefazolin (UWHC)	Resistant	>=32	MIC (UG/ML)	Final
Cefepime (UWHC)	Susceptible	2	MIC (UG/ML)	Final

AST Reporting, Importance of Site, Importance of Guidance in Reporting

- Do not report AST for CSF for drugs that do not reliably cross BBB
- Do not report AST for respiratory specimens with drugs with limited ELF penetration
- Do not report AST for urine specimens for drugs that do not penetrate urine
- Caution in reporting drugs for blood stream infection
  - E.g. Doxy, TMP-sulfa for MSSA/MRSA

- ✓ **Do not report on CSF:** Per CLSI: "Warning": The following antimicrobial agents should not be routinely reported fo isolated from CSF. These antimicrobial agents are not drugs of choice and may not be effective for treating CSF inf caused by these organisms (i.e., the bacteria included in tables indicated).
  These antibiotics are listed in **bold** in these Reporting Rules. It is appropriate to give results for one of the above a requested by Infectious Disease.
  - ✓ agents administered by oral route only, i.e. amox/clav (Augmentin), Cefpodoxime
  - ✓ 1st and 2nd generation cephalosporins, i.e. cefazolin, cefuroxime
  - ✓ cephamycins, i.e. cefoxitin
  - ✓ clindamycin
  - ✓ macrolides, i.e. erythromycin
  - ✓ tetracyclines, i.e. doxycycline, minocycline, tetracycline, and tigecycline
  - ✓ fluoroquinolones, i.e. ciprofloxacin, levofloxacin, moxifloxacin
  - ✓ Certain carbapenems, but not all of them: doripenem, ertapenem, imipenem (not Mero, mero is ok)
- ✓ **Do not report on SPUC/ BALC/Bronch wash (LRT=Lower Respiratory Tract):**
  - ✓ Daptomycin should not be reported
- ✓ **Do not report on Urine:** The following should not be reported, per CLSI unless noted otherwise:
  - ✓ Clindamycin
  - ✓ Erythromycin
  - ✓ Chloramphenicol
  - ✓ Minocycline on Staph
  - ✓ Moxifloxacin on Staph (per CLSI) and Ferms (per FDA and Stewardship)
- ✓ **Antibiotics For Urine Only:** The following are limited to use in treating UTI's, per CLSI unless noted otherwise:
  - ✓ Fosfomycin: For *E. faecalis* and *E. coli* only
  - ✓ Nitrofurantoin
  - ✓ For Enterococcus: Ciprofloxacin, Levofloxacin, and Tetracycline

Monitoring - Tracking and Reporting

- **You can't know how to use antimicrobials most effectively (i.e., stewardship) in your healthcare setting without knowing your drug use, organism epidemiology, and resistance rates!**

We do both, highly recommended but takes resources:

1. Internal tracking and analyses
   - Antimicrobial use monitoring (restricted and unrestricted agents)
   - General resistance patterns and antibiograms
   - Ad hoc resistance evaluation and antibiograms
2. Participate in NHSN (CDC National Healthcare Safety Network) AUR (Antimicrobial Use and Resistance) Module and the State Stewardship collaborative

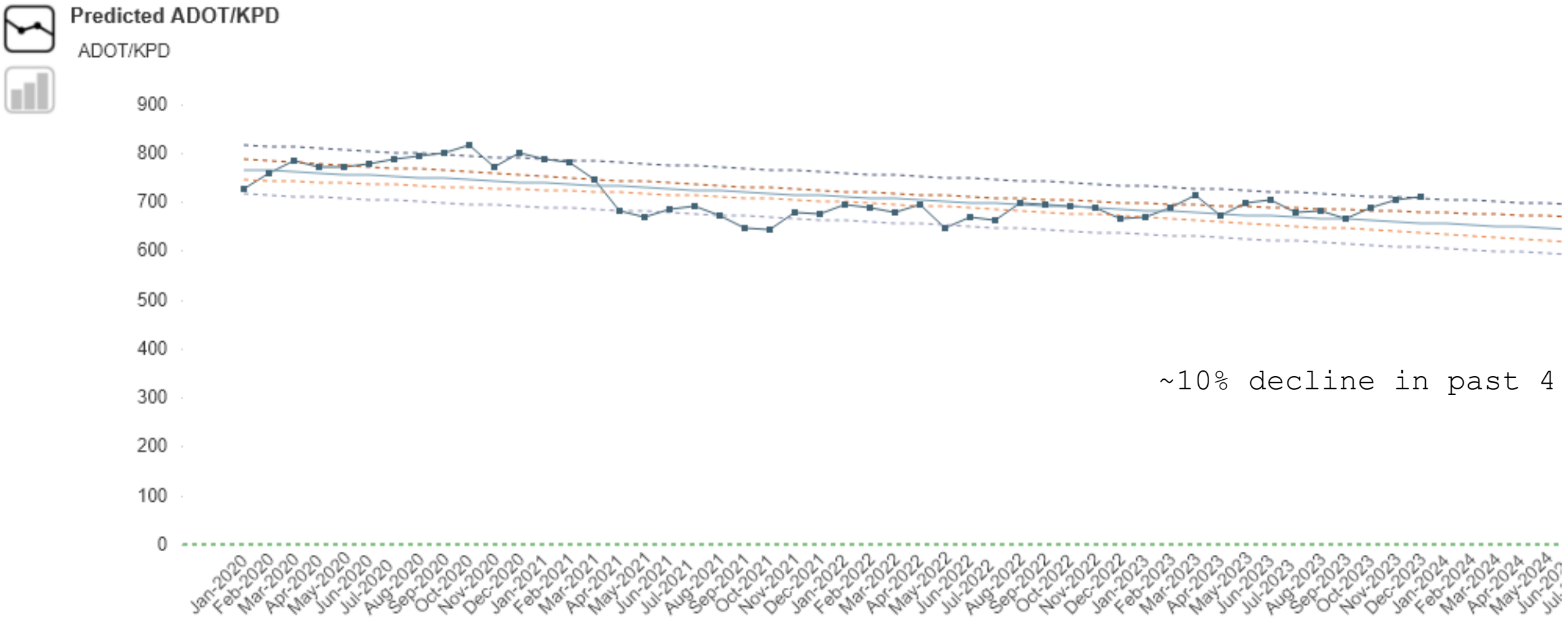
Wisconsin Department of Health Services – Partners to Assist WI Hospitals in Stewardship

- WI DHS supports inpatient facilities with NHSN Antibiotic Use (AU) and Antibiotic Resistance (AR) reporting.
- Inpatient facilities reporting AU data will receive DHS-generated AU reports
- DHS is developing a statewide antibiogram and critical access hospital antibiogram using NHSN AR data and will publish on the DHS website by middle of 2025
- WI DHS sponsors the Wisconsin Collaborative for Healthcare Quality Antibiotic Stewardship Improvement Team, developing outpatient antibiotic use measures for member organizations and supporting an education series.
- WI DHS has published reports of statewide antibiotic use in outpatient and dental settings using

https://www.dhs.wisconsin.gov/antimicrobial-stewardship/index.htm

Overall Antimicrobial Use for UWH Inpatients

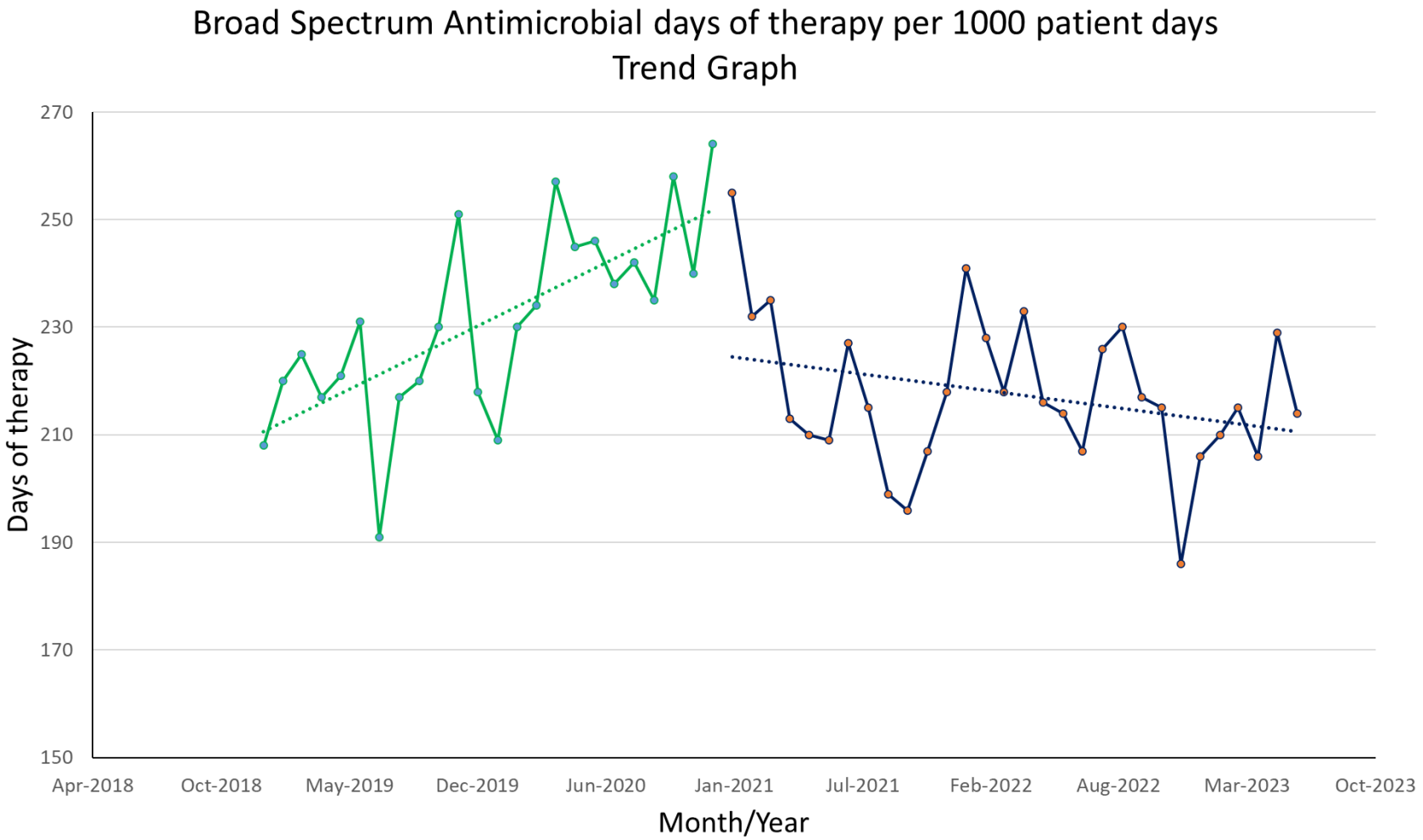
DOT/1000PD Therapy Patients **Predictive DOT/KPD**



~10% decline in past 4 y

Broad Spectrum GNR-active Antimicrobial use for UWH Inpatients

COVID drove much of the upswing



Antibiograms

- What are they?
  - Cumulative report (tabular) of percent susceptible/resistant by organism and drug
- Types?
  - They can be all specimens, site/specimen specific, ward specific, team specific, patient population specific, clinic specific, etc.
  - Very large hospitals often can have many "sub" antibiograms, most community and smaller hospitals often have 1 or 2 (a total antibiogram with perhaps a urine culture specific antibiogram)
- Guidance?
  - Many, CLSI is likely the most often cited
- Are they clinically useful on specific patients?
  - For specific patient use, antibiograms help to inform the clinician of what may be the most appropriate medication to use empirically prior to any microbiology results
- Are they clinically useful to institution?
  - Yes, they help inform on year-to-year changes within the health system on resistance patterns and inform general

Organisms	Isolates	Ampicillin	Sulbactam Amp	Piperacillin/Tazobactam	Cefazolin	Cefuroxime	Cefoxitin	Ceftriaxone	Ceftazidime	Cefepime
(GROUPER) Citrobacter freundii complex	330	—	2 (223) 0	300 (328) 91	—	0 (135) 0	1 (135) 0	258 (329) 78	267 (312) 85	324 (328) 98
(GROUPER) Citrobacter sp. (non-freundii)	371	—	232 (251) 92	369 (371) 99	1 (1) 100	131 (157) 83	151 (157) 96	351 (371) 94	362 (369) 98	366 (371) 98
(GROUPER) Klebsiella/Enterobacter aerogenes	306	—	0 (199) 0	269 (306) 87	0 (1) 0	124 (149) 83	0 (149) 0	150 (200) 75	250 (306) 81	302 (306) 98
(GROUPER) Enterobacter cloacae complex - all species	576	—	0 (381) 0	465 (575) 80	—	102 (321) 31	0 (317) 0	263 (381) 69	422 (575) 73	559 (575) 97
(GROUPER) Escherichia coli	9890	6254 (9889) 63	4548 (6459) 70	9703 (9880) 98	8914 (9885) 90	3239 (3713) 87	3531 (3713) 95	9187 (9890) 92	9310 (9890) 94	9431 (9884) 95
(GROUPER) Klebsiella oxytoca	553	0 (1) 0	239 (351) 68	521 (552) 94	84 (552) 15	235 (272) 86	266 (272) 97	494 (552) 89	522 (552) 94	527 (552) 95
(GROUPER) Klebsiella pneumoniae	1778	0 (2) 0	958 (1160) 82	1719 (1778) 96	1613 (1777) 90	642 (736) 87	695 (737) 94	1651 (1778) 92	1665 (1777) 93	1691 (1776) 95
(GROUPER) Proteus mirabilis	912	781 (912) 85	551 (595) 92	908 (911) 99	814 (912) 89	387 (392) 98	384 (392) 97	902 (912) 98	905 (912) 99	909 (912) 99
(GROUPER) Pseudomonas aeruginosa	1565	—	—	1395 (1560) 89	—	—	—	—	1447 (1563) 92	1484 (1563) 94
(GROUPER) Serratia marcescens	204	—	0 (135) 0	128 (136) 94	—	0 (140) 0	0 (140) 0	181 (204) 88	188 (204) 92	204 (204) 100
(GROUPER) Stenotrophomonas maltophilia	147	—	—	—	—	—	—	—	11 (48) 22	—
(GROUPER) Acinetobacter baumanii/calcoaceticus complex	50	—	47 (50) 94	—	—	—	—	—	43 (50) 86	41 (47) 87
(GROUPER) Acinetobacter baumannii complex	1	—	1 (1) 100	—	—	—	—	—	0 (1) 0	0 (1) 0
(GROUPER) Enterobacteriaceae/Enterobacterales	14348	6913 (10621) 65	6255 (9393) 66	13850 (14270) 97	10995 (12562) 87	4602 (5735) 80	4837 (5736) 84	12931 (14052) 92	13359 (14303) 93	13734 (14296) 96

Limitations to Antibiogram

- **Data do not take into account patient factors such as history of infection or past antimicrobial use, nor if patient has had resistant pathogens previously that would clearly impact empirical choices.**
  - **Resistance patterns for certain drugs vary significantly by age, and a patient's underlying medical condition may affect how well an antimicrobial works.**
- **Does not differentiate community acquired versus nosocomial infection**
- **Impacted by culturing practices at facility/amongst clinicians**
  - **Highly impacted by decision to limit to first isolate per patient per analysis period (only ~50% of hosp do this, and what analysis period to use is debatable)**
- **Does not include PK factors, site (often), severity such that not all options listed in the antibiogram may be appropriate for a clinical situation**
- Need to have at least 30 isolates for significance
- Data are the result of single organism-antimicrobial combinations, therefore do not show trends in cross-resistance of an organism to other drugs, nor do they reveal synergistic properties of antimicrobials used in combination

Outpatient Inpatient Outpatient Inpatient Only Positive Bloods

Organisms	Piperacillin/Tazobactam	Piperacillin/Tazobactam	Ceftriaxone	Ceftriaxone	Ceftriaxone
(GROUPER) Citrobacter freundii complex	129 (137) 94	92 (107) 85	108 (138) 78	75 (107) 70	5 (7) 71
(GROUPER) Citrobacter sp. (non-freundii)	175 (175) 100	68 (70) 97	171 (175) 97	57 (70) 81	2 (2) 100
(GROUPER) Klebsiella/Enterobacter aerogenes	96 (106) 90	71 (88) 80	55 (74) 74	38 (59) 64	3 (4) 75
(GROUPER) Enterobacter cloacae complex - all species	187 (216) 86	166 (230) 72	106 (140) 75	102 (167) 61	18 (31) 58
(GROUPER) Escherichia coli	3967 (4021) 98	1421 (1472) 96	3727 (4024) 92	1292 (1475) 87	185 (231) 80
(GROUPER) Klebsiella oxytoca	196 (210) 93	167 (185) 90	186 (210) 88	156 (185) 84	18 (26) 69
(GROUPER) Klebsiella pneumoniae	715 (734) 97	447 (475) 94	689 (734) 93	414 (475) 87	53 (64) 82
(GROUPER) Proteus mirabilis	416 (417) 99	210 (210) 100	415 (417) 99	206 (210) 98	20 (20) 100
(GROUPER) Pseudomonas aeruginosa	504 (539) 93	565 (681) 82	—	—	—
(GROUPER) Serratia marcescens	45 (47) 95	60 (63) 95	63 (69) 91	74 (87) 85	9 (12) 75
(GROUPER) Stenotrophomonas maltophilia	—	—	—	—	—
(GROUPER) Acinetobacter baumanii/calcoaceticus complex	—	—	—	—	—
(GROUPER) Acinetobacter baumannii complex	—	—	—	—	—
(GROUPER) Enterobacteriaceae/Enterobacterales	5712 (5845) 97	2528 (2704) 93	5322 (5761) 92	2242 (2645) 84	310 (396) 78

Rapid Diagnostic Tests (RDT)

- Highly recommend multi-d group with stakeholders from clinical and non-clinical areas to discuss, prioritize, evaluate, implement, re-evaluate
- Syndromic Tests
  - Rapid Respiratory virus identification
  - Rapid Blood culture pathogen identification
  - Rapid Respiratory (Pneumonia) pathogen identification
  - Rapid Gastrointestinal pathogen identification
  - Rapid Meningitis pathogen identification
  - Rapid Septic arthritis pathogen identification
- Almost every study that has looked at clinical effects of RDT have shown alone, they have limited impact on patient care, but when combined with an ASP program, have dramatic impacts on appropriate therapy, de-escalation of therapy, discontinuing inappropriate therapy, time to optimal therapy, avoidance of admission, and decreased length of stay

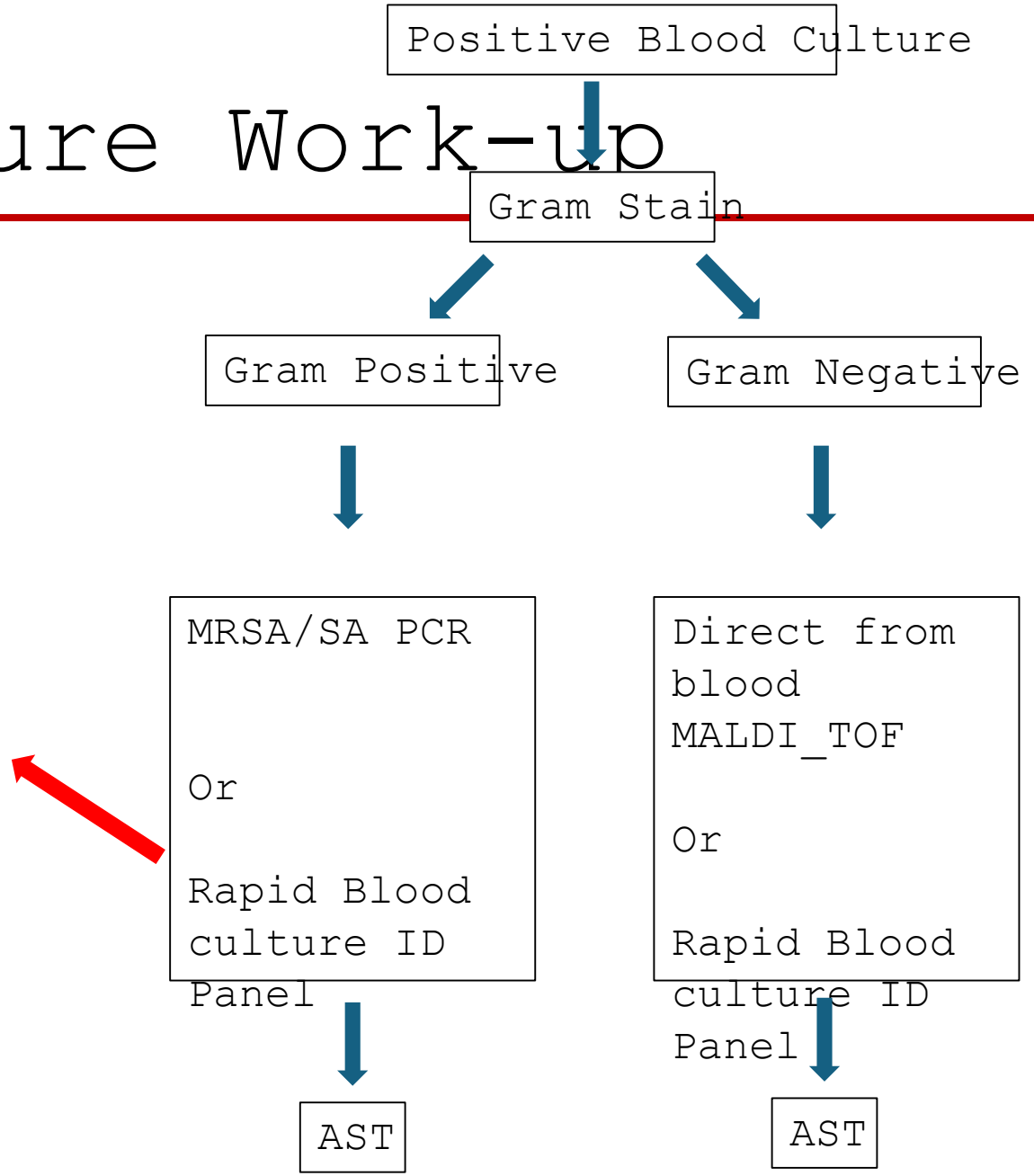
"Our technical capabilities are exceeding our ability to apply them effectively and economically to human problems"

Rapid Respiratory Virus Testing

- The #1 cause of over-/inappropriate prescribing of antimicrobials in ambulatory setting is due to URI, of which the vast majority are viral in nature
- The test needs to be available, timely, actionable
  - E.g. Urgent care center experience at UWH
- Range of targets -> Limited – Full panel testing (highly rec the latter)
  - Flu only, Flu/COVID-19, Flu/COVID-19/RSV
  - Flu/COVID-19/RSV/hMPV/PI/Entero/Rhino/Adeno/sCOR
    - One commercial test available that is CLIA-waived, POCT
- Most use NP sampling

Rapid Blood Culture Work-up

- The rapid detection of genus, species, and resistance determinants is critically important in sepsis
- Many commercial platforms that can quickly identify (within hours) from a positive blood culture bottle the specific pathogen
- Limitations
  - Cost
  - Rapid phenotypic characterization is still a work in progress, with only one commercial system
  - Just because a resistance determinant is not molecularly found does not mean resistance to that drug is not present (e.g., ESBL)
    - In other words, positive predictive value is excellent and can provide timely information to escalate/modify antimicrobial coverage, but negative



Common Commercial Platforms	Technology	Pathogen Detection	Notes	Resistance Detection
Xpert MRSA/SA BC	Multiplex NA amplification	2	SA ID only (MRSA/MSSA)	mecA
Verigene BC	DNA Microarray	20	Separate GP and GN panels	mecA, VanA, VanB CTX-M, KPC, IMP, VIM, NMD, OXA
Biofire FilmArray BCID2	Multiplex NA amplification	32	GP/GN/Yeast all in one cartridge	mecA, mecC, MREJ, VanA, VanB CTX-M, KPC, OXA-48 like, IMP, NDM, VIM, mcr1
ePlex BCID	Multiplex NA amplification and hybridization	56	Separate GP, BN, fungal (yeast) panels	mecA, mecC, VanA, VanB CTX-M, KPC, OXA (-48 and -23), IMP, NDM, VIM
T2 biosystems	Magnetic Resonance	10	Bacterial (limited) and candida panels	T2 resistance panel (RUO) – mecA, vanA, vanB, CTX-M, AmpC, KPC, OXA-48, NDM/VIM/IMP
MALDI-TOF (direct from Blood Culture)	Mass spec	Unlimited GNR	Only done direct from blood culture on GN, requires some manual technician expertise or	none

Blood Culture ID 1 by BioFire PCR [392935334] (Abnormal)

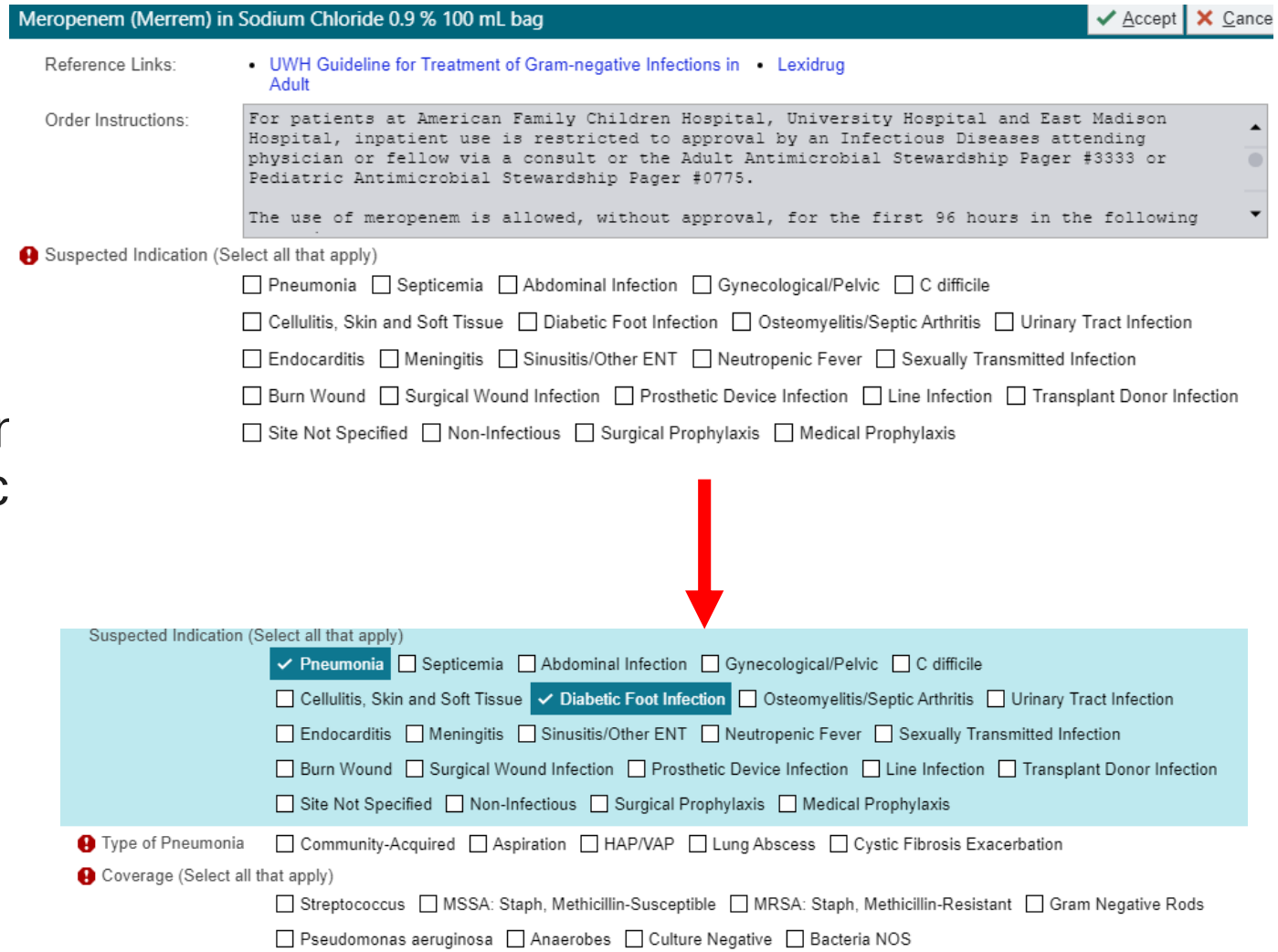
Lab Status: Final result

Specimen: Blood

Interpretation of Blood PCR:	--
Comment: Results suggest Streptococcus pyogenes (group A Strep)	
Enterococcus faecalis	Not Detected
Enterococcus faecium	Not Detected
vanA/B	N/A
Comment:	
mecA/C and MREJ (MRSA)	N/A
Comment:	
Staphylococcus aureus	Not Detected
Staphylococcus spp.	Not Detected
mecA/C	N/A
Comment:	
Staphylococcus epidermidis	Not Detected
Staphylococcus lugdunensis	Not Detected
Streptococcus spp.	Detected !
Streptococcus agalactiae (Group B)	Not Detected
Streptococcus pneumoniae	Not Detected
Streptococcus pyogenes (Group A)	Detected !
Acinetobacter calcoaceticus-baumannii complex	Not Detected
Pseudomonas aeruginosa	Not Detected
Stenotrophomonas maltophilia	Not Detected
Enterobacterales	Not Detected
CTX-M	N/A
Comment:	
Escherichia coli	Not Detected
Klebsiella aerogenes	Not Detected
Klebsiella oxytoca	Not Detected
Klebsiella pneumoniae group	Not Detected
Enterobacter cloacae complex	Not Detected
Proteus spp.	Not Detected
Salmonella spp.	Not Detected
Serratia marcescens	Not Detected
IMP	N/A
Comment:	
KPC	N/A
Comment:	
mcr-1	N/A
Comment:	
NDM	N/A
Comment:	
OXA-48-like	N/A
Comment:	
VIM	N/A
Comment:	
Bacteroides fragilis	Not Detected
Haemophilus influenzae	Not Detected
Listeria monocytogenes	Not Detected
Neisseria meningitidis	Not Detected
Candida albicans	Not Detected
Candida auris	Not Detected
Candida glabrata	Not Detected
Candida krusei	Not Detected
Candida parapsilosis	Not Detected
Cryptococcus neoformans/gattii	Not Detected
Candida tropicalis	Not Detected

Other Stewardship Principles/Activities

- All antimicrobial orders require an indication
- Beta-lactam allergy management
- Antibiotic timeouts
  - The CDC and The Joint Commissior recommend performing an Antibiotic Timeout 48-72 hours after starting empiric antibiotics to reassess their necessity. This ensures antibiotics are appropriately dosed, de-escalated when possible, and the right antibiotics are used.
- Drug shortage mitigation
  - We have managed 35 since 2022



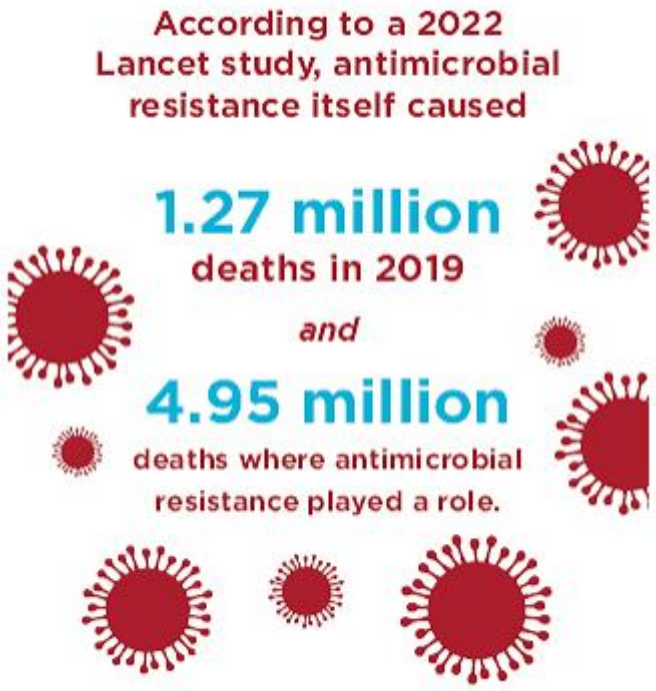
Communicate, Educate, Leverage ID

- I couldn’t conclude a talk about stewardship without mentioning that communication is key. Talk to everyone, include stakeholders, leverage the expertise of all areas – Infectious disease physician, infectious disease pharmacist, general clinicians (surgical and medical), general pharmacy, lab, IS, regulatory specialists, reporting specialists, infection control, etc.
- Regular informal meetings with groups (i.e., attending division meetings, chalk talks, etc.) and formal talks (“updates in xyz”, grand rounds, etc.) are invaluable methods to educate on changes within antimicrobial stewardship and maintain healthy collaborative relationships
- Infectious Disease physicians and pharmacists want to help

Conclusions

CDC 2019

Lancet 2022



- Stewardship takes expertise (ID), a community of stakeholders, of which the lab is an integral part, and resources with a common goal of working collaboratively to optimize and preserve antimicrobial therapy
- Successful stewardship involves many processes, when done well the effects on improvement in patient care, decreased complications, slowing resistance, preservation of antimicrobials, and decreased costs are substantial
- In the face of increasing resistance and dry pipeline, stewardship is an essential asset we can readily deploy to mitigate the effect of infectious diseases on so many areas where we have made such impressive advances
  - Oncology, Immunology, Rheumatology, GI, Transplant, Surgeries (orthopedic, etc.)
  - The ability to improve quality of life and length of life for patients with severe medical and surgical obstacles is challenged chiefly by our ability to mitigate infections from antimicrobial resistant pathogens

Thank you

ajlepak@medicine.wisc.edu

Troubleshooting AST Verification/Validation Issues

Megan Selle MLS(ASCP)^{CM}
Laboratory Supervisor, Microbiology, ThedaCare

Alana Sterkel, PhD, D(ABMM), SM(ASCP)^{CM}
Associate Director, Communicable Diseases, WSLH
Assistant Professor, UW Madison

Clicker Question #1

What is your experience with AST Validations or Verifications?

A. Validation Pro, I could teach this!
B. I’ve been around the block
C. I’ve done a little or helped others
D. Newbie eager to learn!

Clicker Question #2

Which of these most closely matches your current role?

A. Lab Director
B. Lab Manager/Supervisor
C. Lab AST specialist
D. Lab Bench Technologist
E. Non-laboratorian

A Guide to Validation Plans

- CLSI requirements for an AST validation {cite CLSI docs}
  - If you haven't attended WSLH's previous discussions on breakpoint changes, I highly recommend checking out the AST Validation Webinars and worksheets provided by CLSI (https://clsi.org/meetings/ast/breakpoints-in-use-toolkit/)
- Differences between AST and other validations
  - AST validations have become very complicated. Most test system validations are set for the life of the test system if you keep the same test system and there are no major upgrades that change the way the instrumentation/test functions.
  - Any AST system is now subject to a major breakpoint validation every time the breakpoints are updated, even though nothing has changed with the instrument or test method.

Validation Definitions

- Essential vs categorical agreement (see CLSI toolkit for breakdown of calculations)
  - Essential agreement (EA): MIC result obtained with the antimicrobial susceptibility testing system that is within one doubling dilution step for bacteria (and two for yeast)
  - Categorical agreement (CA): agreement of susceptible, intermediate, susceptible-dose dependent and resistant results between a breakpoint test or a MIC test and the reference method.
- Error Categories:
  - Minor error (mE): difference in test results between a new antimicrobial susceptibility testing system and reference AST where one result is intermediate and the other is susceptible or resistant
  - Major Error (ME): error when the reference method result is susceptible and the antimicrobial susceptibility testing system under evaluation is resistant
  - Very Major Error (VME): error when the reference method result is resistant and the result from the antimicrobial susceptibility testing system under evaluation is susceptible
- Reproducibility requirements:
  - A minimum of 5 isolates (either QC or clinical strains) should be tested 3 times each
  - 95% of results should be within essential agreement or within the QC specifications

Same Test System, New Regulations- A Case Study

- The best laid plans…
- Gather up your known materials, the handy CLSI M52 Document, CLSI validation kit, CDC AR Bank isolates complete with images
- All set… right?

• Photo retrieved from: https://www.istockphoto.com/search/2/image-film?phrase=road+map

Disclaimer

- This is a case study of only one clinical laboratory. The steps taken at this laboratory may not be suited for every laboratory and is up to laboratory director discretion.

Picture derived from: https://makeameme.org/meme/disclaimer-this

Plans

Part C. BIT Summary Template

☐ Verification of ☑ Validation of Gentamicin, Tobramycin, Piperacillin/Tazobactam Ciprofloxacin **Breakpoints for (organism/organism group)** Enterobacterales

tested by (AST Method) Biomerieux Vitek 2 AST-GN79

Studies performed (dates): 5/17/23, 8/2/23

I. Purpose

☐ Verify or ☑ Validate performance of (Name of Method or Commercial AST Device) Biomerieux Vitek 2 AST-GN79

For ☐ organism or ☑ organism group Enterobacterales

Reference/Comparator results from (see NOTE below, II.B.) AR Isolate Bank

For Antimicrobial(s) and Breakpoint Values

	Old Breakpoints (MIC µg/ml)				New Breakpoints (MIC µg/ml)				
Antimicrobial(s)	S	SDD	I	R	S	SDD	I	R	Breakpoint Source (FDA/CLSI)
Gentamicin	<=4		8	>=16	<=2		4	>=8	CLSI 2023
Tobramycin	<=4		8	>=16	<=2		4	>=8	CLSI 2023
Piperacillin/Tazobactam	<=16		32-64	>=128	<=8	16		>=32	CLSI 2023
Ciprofloxacin	<=1		2	>=4	<=0.25		0.5	>=1	CLSI 2023

Abbreviations: I, intermediate; MIC, minimal inhibitory concentration; R, resistant; S, susceptible; SDD, susceptible dose dependent.

II. Verification/Validation Study

A. AST System

Panel/Card AST-GN79 Software version 9.02

B. Accuracy

Number of isolates 30

Isolate source(s) AR Isolate Bank- CRE/IMP Panels

(eg, CDC & FDA Antibiotic Resistance (AR) Isolate Bank, clinical isolates)

Reference result source(s) AR Isolate Bank Established MIC

(eg, CDC & FDA AR Isolate Bank MICs, in-house reference broth microdilution, reference laboratory)

NOTE: Reference result may be obtained from parallel testing using a reference AST method or comparator AST me
for the new breakpoints or preestablished using a reference (eg, CDC & FDA AR Isolate Bank) or verified/validated c

C. Reproducibility (precision)

Number of isolates 60 (4 drugs tested 3 times a day for 5 days)

Isolate source(s) Gram negative QC ATCC Strains

(eg, CDC & FDA AR Isolate Bank, clinical isolates quality control strains)

Number of replicates 3 times per day for 5 days

D. Quality Control

Isolate(s) E. coli ATCC 25922, E. coli ATCC 35218 Testing frequency 3 times a day for 5 days

(ie, name/strain number) (eg, per run)

E. Analysis

1. Interpret MIC results manually utilizing new breakpoints as listed above (see I. Purpose).
2. Compare interpretive category results (eg, S, SDD, I, R) obtained from test system to the interpretive category obtained from the reference/comparator results.
3. General guidance for acceptable **accuracy**

Categoric Agreement (CA)	≥90%
Very Major Errors (VME)	<3%
Major Errors (ME)	<3%
Minor Errors (MiE)	Determined by the laboratory director.

4. **Note:** A category agreement of <90% may be acceptable if the majority of errors are minor and the minor errors have essential agreement (ie, within ±1 two-fold dilution).
5. Acceptable **reproducibility**
   95% of replicate results for a single antimicrobial agent/organism fall into either an S, I, SDD, or R category.

III. Procedure

A. Materials and testing procedure for system to be verified/validated

Described in SOP Validation and Implementation Guidelines (this Laboratory's SOP #)

B. Record results on **Appendix E2**

The Best Laid Plans

- Accuracy, CLSI guidelines (M52):
  - Categorical agreement (CA): >=90%
  - Very Major Errors (VME): <3%
  - Major Errors (ME): <3%
  - Minor Errors (mE): Determined by laboratory director
- But I'm validating numbers (MICs) around a breakpoint that is changing the category… so my categorical agreement is going to look pretty bad…
  - Categorical agreement <90% may be acceptable if majority of errors are minor and the minor errors have essential agreement (EA)- within +/- 2 fold dilution.

Sometime Fail

All highlighted blue and yellow areas are either categorical or essential agreement failures

Isolates tested 5/17/23

Part 1 2023 CLSI Breakpoint Validations- Enterobacterales

Accession Number	Hyde MIC/Interpretation					Jekyll MIC/Interpretation					Original Run Breakpoints				
	Amikacin	Gentamicin	Tobramycin	Pip/Tazo	Ciprofloxacin	Amikacin	Gentamicin	Tobramycin	Pip/Tazo	Ciprofloxacin	Amikacin	Gentamicin	Tobramycin	Pip/Tazo	Ciprofloxacin
(CRE Iso Bank) SAMN04014953, AR-0112, K. pneumoniae	≥64,R	≥16,R	≥16,R	≥128,R	≥4,R						16, R *	16, R	16, R	128, R	8, R
(CRE Iso Bank) SAMN04014954, AR-0113, K. pneumoniae	≥64,R	≥16,R	≥16,R	≥128,R	≥4,R						32, R	>=16, R	>=16, R	>=128, R	>=8, R
(CRE Iso Bank) SAMN04014955, AR-0114, E. coli						≤2,S	4,I	8,R	≥128,R	≥4,R	8, I	8, R	8, R	>=128, R	>=8, R
(CRE Iso Bank) SAMN04014958, AR-0117, K. pneumoniae						≥64,R	≥16,R	≥16,R	≥128,R	≥4,R	16, R *	8, R	>=16, R	>=128, R	8, R
(CRE Iso Bank) SAMN04014961, AR-0120, K. pneumoniae						≥64,R	≥16,R	≥16,R	≥128,R	≥4,R	32, R	16, R	16, R	>=128, R	>=8, R
(CRE Iso Bank) SAMN04014969, AR-0128, E. coli						≥64,R	≥16,R	≥16,R	≥128,R	≥4,R	>=64, R	>=16, R	>=16, R	128, R	>=8, R
(CRE Iso Bank) SAMN04014975, AR-0134, Raoultella ornithinolytica						≤2,S	8,R	≥16,R	≥128,R	≤0.25,S	2, S	4, I	16, R	64, R	<=0.25, S
(CRE Iso Bank) SAMN04014977, AR-0136, E. cloacae						≤2,S	8,R	≥16,R	≥128,R	≥4,R	2, S	4, I	16, R	>=128, R	>=8, R
(IMP Iso Bank) SAMN28842366, AR-1109 E. cloacae cpx	≤2,S	4,I	8,R	≥128,R	≥4,R						<=1, S	4, I	4, I	>128, R	>8, R
(IMP Iso Bank) SAMN28842368, AR-1111 Providencia rettgeri	≤2,S	4,I	4,I	≤4,S	2,R						<=1, S	NT	2, S	<=4, S	2, R
(IMP Iso Bank) SAMN28842374, AR-1117 P. mirabilis						≤2,S	≤1,S	≤1,S	≤4,S	≤0.25,S	4, S	2, S	2, S	<=4, S	<=0.25, S
(CRE Iso Bank) SAMN04014956, AR-0115, K. pneumoniae						≤2,S	8,R	≥16,R	≥128,R	≥4,R	<=1, S	8, R	16, R	>128, R	>8, R
(CRE Iso Bank) SAMN04014957, AR-0116, C. freundii						≤2,S	≥16,R	≥16,R	≥128,R	≥4,R	2, S	16, R	16, R	>128, R	>8, R
(CRE Iso Bank) SAMN04014959, AR-0118, E. coli						≥64,R	≥16,R	≥16,R	≥128,R	≥4,R	>64, R	>16, R	>16, R	>128, R	>8, R
(CRE Iso Bank) SAMN04014960, AR-0119, E. coli						≥64,R	≥16,R	≥16,R	≥128,R	≥4,R	>64, R	>16, R	>16, R	>128, R	>8, R
(CRE Iso Bank) SAMN04014962, AR-0121, S. marcescens						≤2,S	≤1,S	≤1,S	NT	≤0.25,S	2, S	0.5, S	1, S	<=4, S	<=0.25, S
(CRE Iso Bank) SAMN04014985, AR-0144, Kluyvera ascorbata						≤2,S	8,R	≥16,R	≥128,R	2,R	8, I	8, R	16, R	>128, R	2, R
(CRE Iso Bank) SAMN04014986, AR-0145, K. pneumoniae	16,R	≤1,S	≥16,R	≥128,R	≥4,R						16, R	1, S	16, R	>128, R	>8, R
(CRE Iso Bank) SAMN04014987, AR-0146, K. pneumoniae	16,R	≤1,S	≥16,R	≥128,R	≥4,R						32, R	1, S	16, R	>128, R	>8, R
(CRE Iso Bank) SAMN04014988, AR-0147, K. oxytoca						≤2,S	8,R	8,R	≥128,R	≤0.25,S	<=1, S	4, I	8, R	16, SDD	<=0.25, S
(CRE Iso Bank) SAMN04014989, AR-0148, K. pneumoniae						≥64,R	≥16,R	≥16,R	≥128,R	≥4,R	>64, R	>16, R	>16, R	>128, R	>8, R
(CRE Iso Bank) SAMN04014997, AR-0156, P. mirabilis						4,S	2,S	≥16,R	≤4,S	2,R	8, I	4, I	>16, R	<=4, S	4, R
(CRE Iso Bank) SAMN04014998, AR-0157, Citrobacter spp.						≥64,R	≥16,R	≥16,R	≥128,R	≥4,R	>64, R	>16, R	>16, R	>128, R	>8, R
(CRE Iso Bank) SAMN04014999, AR-0158, K. pneumoniae						≤2,S	≥16,R	8,R	≥128,R	≥4,R	<=1, S	>16, R	8, R	>128, R	>8, R
(CRE Iso Bank) SAMN04015000, AR-0159, P. mirabilis						8,I	≥16,R	≥16,R	8,S	2,R	>64, R	>16, R	>16, R	128, R	2, R
(CRE Iso Bank) SAMN04015001, AR-0160, K. pneumoniae						≤2,S	≤1,S	≤1,S	≥128,R	≤0.25,S	<=1, S	0.5, S	<=0.5, S	>128, R	<=0.25, S
(CRE Iso Bank) SAMN04015002, AR-0161, K. aerogenes	≤2,S	≥16,R	8,R	≥128,R	≤0.25,S						2, S	>16, R	16, R	>128, R	<=0.25, S
(CRE Iso Bank) SAMN04015003, AR-0162, E. coli	≤2,S	≤1,S	≤1,S	≥128,R	≥4,R						2, S	1, S	<=0.5, S	>128, R	>8, R
(CRE Iso Bank) SAMN04015004, AR-0163, E. cloacae	8,I	≥16,R	≥16,R	≥128,R	≥4,R						4, S	>16, R	>16, R	>128, R	>8, R
(CRE Iso Bank) SAMN04015005, AR-0164, E. cloacae	≤2,S	≤1,S	≤1,S	≥128,R	≤0.25,S						<=1, S	<=0.25, S	<0.5, S	>128, R	<=0.25, S

Tested 8/2/23

MICs in RED- Have an affected breakpoint
*Isolates are already tested an in Vitek- reanalyze
**Isolates are already in Vitek and a backup isolate
Patient 6 and 12 did not grow, added Patient 23 and 24

Gent 83% | Tob 93% | Cip 93% ME: P/T-1

VME: * P/T + Cipro MiE: Ami: Gent: Tob: & P/T Cip

* Note- validation study initially performed 5/17/23, before the CLSI toolkit came out. We used patient isolates previously tested on the Vitek. We ~~before~~ retested some isolates, re-analyzed some after breakpoints were changed. Did not perform precision study because we were told this wasn't necessary as we did this during instrument validation. We performed precision study * CDC isolates later (8/2/23) because clinical specimens weren't tested w/ manual micro broth dilutions.

https://tenor.com/search/head-scratcher-gifs

Now What?

- Gentamicin:
  - 5 categorical agreement discrepancies (all w/in +/- 2 fold dilution), minor error rate of 16.7%, categorical agreement was 83.3%, our validation acceptability is 90%.
    - All minor errors had essential agreement
- Piperacillin/Tazobactam
  - 1 VME- CDC MIC reported 128, R, clinical lab tested MIC was 8, S
  - 1 ME- CDC MIC reported 16, SDD, clinical lab tested MIC was >128, R
  - No minor errors
- Tobramycin and Ciprofloxacin
  - Met all validation standards, no issues

Clicker Question #3

What are the next steps to resolve the piperacillin/tazobactam discrepancies?

A. Add more isolates to the study to “dilute out” the errors.
B. Test the discrepant isolates in triplicate.
C. Send isolates to a tie breaker lab.
D. Give up and go home.

Poll the Resources

- Test the failed isolates in triplicate- is it us, is it them?

Part 1 2023 CLSI Breakpoint Validations- Enterobacterales

	Re-Run Results														1st Run Results					Original Run Breakpoints				
Accession Number/Repeated ID	Amikacin #1	Amikacin #2	Amikacin #3	Gentamicin #1	Gentamicin #2	Gentamicin #3	Tobramycin #1	Tobramycin #2	Tobramycin #3	Pip/Tazo #1	Pip/Tazo #2	Pip/Tazo #3	Cipro #1	Cipro #2	Cipro #3	Amikacin	Gentamicin	Tobramycin	Pip/Tazo	Ciprofloxacin	Amikacin	Gentamicin	Tobramycin	Pip/Tazo	Ciprofloxacin
(CRE Iso Bank) AR-0159 VME										8, S	8, S	8, S				8, I	>=16, R	>=16, R	8, S	2, R	>64, R	>16, R	>16, R	128, R	2, R
(CRE Iso Bank) AR-0147 ME										64, R	>=128, R	>=128, R				<=2, S	8, R	8, R	>=128, R	<=0.25, S	<=1, S	4, I	8, R	16, SDD	<=0.25, S

VME

ME

- Well that’s not going to help this situation… what next (at least our system is consistent)

3rd Attempt is the Ticket?

- Send the isolates out to the reference lab as a referee:
  - VME (AR-0159) tested at a reference lab as >/=128, R
    - Discrepancy not resolved
  - ME (AR-0147) tested at a reference lab as >/=128, R
    - Discrepancy resolved, matched what clinical lab had also reported

Troubleshooting 2.0

- Vendor support:
  - Verify the organism was subbed out twice before testing ✓
  - Repeat testing on a different instrument ✓
  - Send isolate to other laboratories with same card/instrumentation
- CAP: discontinue piperacillin/tazobactam testing or use an alternate method to confirm piperacillin/tazobactam results
  - Major problem, one of the most important Gram negative antibiotics for inpatient care
  - Performing an alternate method for pip/tazo for Enterobacterales spp. would be expensive and time consuming
- The lab went with the vendor plan

Troubleshooting 2.0

- Sent our VME isolate out to 2 different labs that had the similar antibiotic card and instrument, the results:
  - Lab 1: piperacillin/tazobactam 8, S (AES database deduced isolate as R)
  - Lab 2: piperacillin/tazobactam 8, S

- Vindication?

Clicker Question #4

What would you do?

A. Accept the validation and move on.
B. Perform more testing and add more specimens to the validation.
C. I'd have to defer to the lab director, I don't know.

ASM- The Voice of Reason

- A podcast was given by ASM: Susceptibility Testing for Piperacillin-Tazobactam (https://asm.org/Podcasts/Editors-in-Conversation/Episodes/Susceptibility-Testing-for-Piperacillin-Tazobactam?sr_id=b0d2e3d2-bb61-4e00-9f94-ea0f1918e655&sr_pos=0)
  - 1 isolate failing validation must be taken into context and piperacillin/tazobactam is too important of a drug to not change the breakpoints or not report.

What Was the Outcome

- Data Recap:
  - Gentamicin:
    - CA 83.3%
    - mE 16.7%
  - Tobramycin:
    - CA 93.3%
    - mE 6.7%
  - Piperacillin/Tazobactam
    - CA 93.1%
    - VME resolved according to manufacturer
  - Ciprofloxacin
    - CA 100%

What Was the Outcome

- The laboratory director agreed went with ASM guidance and accepted the VME as resolved.
- The updated breakpoints were put into use.
- This validation started in May of 2023, was not resolved and live until November of 2023 due to all of the troubleshooting, repeating samples, finding different labs to send isolates to and the IT build.

Validation Woes and Troubleshooting

Dr. Alana Sterkel

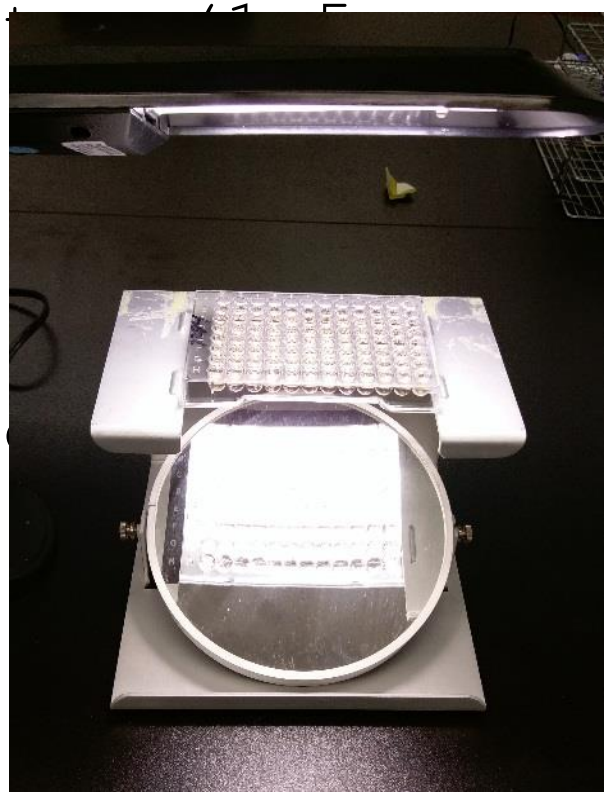
Why Did We Do It?

- Highly drug resistant *Candida auris* is spreading across the globe.
- Testing of clinical isolates for patient care and surveillance is needed.
- The CDC provides a microbroth dilution panel (Trek) for the 7 regional Antimicrobial Resistance Laboratory Network Labs.

- **Goal**: Validate the Trek plates using CDCs protocol for *C. auris* and other *Candida* species

The Panel- YCML3FCAN

- Pre-filled with liquid (100uL), shipped frozen
- Fresh yeast prepared and added to pla
  10^6 cfu/ml)
- Autofill (Sensititer)
- Incubate at 35 C for 18-24 hours
- Manual read with a mirror box, no col
  indicator



Validation Plan

- Reproducibility
  - Panel of 5 isolates tested by 3 different people
- Precision
  - Control strain tested 15 times
- Accuracy
  - Range of MICs for each bug/drug combination

- QC - *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258
- All validation isolates acquired through the CDC AR Isolate Bank

Validation Criteria

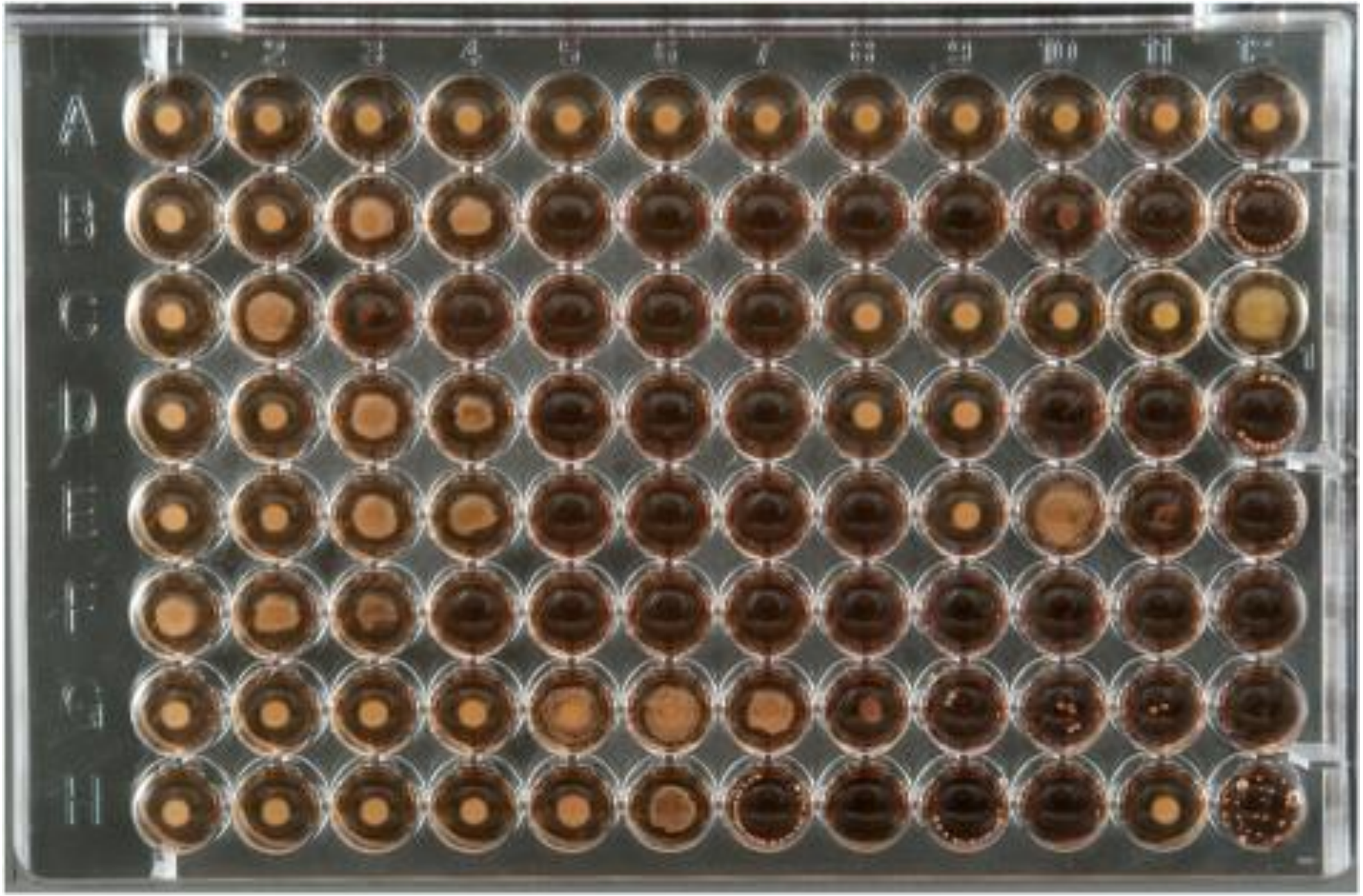
Minor errors	<10% of isolates
Major errors	<3% of the susceptible isolates
Very major errors	<3% of the resistant isolates
Total % categorical agreement	>90% agree and <3% very major errors
Total % essential agreement	<3% of all results

CLSI M23, M27, M45, M60

Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
Voriconazole	0.008	0.016	0.0312	0.063	0.13	0.25	0.5	1	2	4	8	16
Anidulafungin	0.008	0.016	0.0312	0.063	0.13	0.25	0.5	1	2	4	8	16
Caspofungin	0.008	0.016	0.0312	0.063	0.13	0.25	0.5	1	2	4	8	16
Fluconazole	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256
Itraconazole	0.008	0.016	0.0312	0.063	0.13	0.25	0.5	1	2	4	8	16
Isavuconazole	0.004	0.008	0.0156	0.031	0.06	0.13	0.25	0.5	1	2	4	8
Posaconazole	0.008	0.016	0.0312	0.063	0.125	0.25	0.5	1	2	4	8	16
Micafungin	0.008	0.016	0.0312	0.063	0.125	0.25	0.5	1	2	4	8	**POS**


Contrast 10%



QC Isn't Passing!

Passed QC
Low
High

Candida krusei

	Fluconazole	Voriconazole	Posaconazole	Itraconazole	Caspofungin	Anidulafungin	Micafungin	Isavuconazole
Range	8-64	0.06-0.5	0.06-0.5	0.12-1	0.12-1	0.03-0.12	0.12-0.5	0.06-0.5
1	8	0.06	0.015	0.06	0.06	0.015	0.06	0.03
2	8	0.06	0.060	0.12	0.12	0.03	0.06	0.12
3	2	0.03	0.060	0.12	0.25	2	1	0.06
4	2	0.03	0.030	0.12	0.25	1	1	0.03
5	16	0.12	0.03	0.12	0.06	0.015	0.06	0.12
6	16	0.06	0.03	0.12	0.06	0.03	0.06	0.06
7	32	0.12	0.06	0.25	0.12	0.03	0.12	0.12
8	16	0.12	0.12	0.5	0.12	0.015	0.12	0.12

QC isolates from CDC weren't working, ordered fresh from ATCC

QC Isn't Passing!

Candida parapsilosis

	Fluconazole	Voriconazole	Posaconazole	Itraconazole	Caspofungin	Anidulafungin	Micafungin	Isavuconazole	
Range	0.5-4	0.016-0.12	0.03-0.25	0.06-0.5	0.25-1	0.25-2	0.5-2	0.015-0.06	
1	2	0.030	0.015	0.12	0.06	1	0.5	0.03	**DP Lot:17412**
2	0.5	0.015	0.008	0.06	0.06	0.5	0.5	0.015	**DP Lot:17412**
3	2	0.03	0.06	0.12	0.25	1	1	0.03	DP Lot:18105
4	2	0.03	0.06	0.25	0.25	1	1	0.06	DP Lot:18105

Passed QC

Low

High

- A lot of plates we received was bad
- Caspofungin degraded quickly- eventually dropped from the panel

Too Many Errors

			Fluconazole						Voriconazole						Posaconazole						Itraconazole						Caspofungin						Anidulafungin						Micafungin						Isavuconazole						Amphotericin B					
			CDC		WSLH				CDC		WSLH				CDC		WSLH				CDC		WSLH				CDC		WSLH				CDC		WSLH				CDC		WSLH				CDC		WSLH				CDC		WSLH			
			MIC	INT	DP	AV	AB		MIC	INT	DP	AV	AB		MIC	INT	DP	AV	AB		MIC	INT	DP	AV	AB		MIC	INT	DP	AV	AB		MIC	INT	DP	AV	AB		MIC	INT	DP	AV	AB		MIC	INT	DP	AV	AB		MIC	INT	DP	AV	AB	
5/18/2018	0314	Candida glabrata	64	R	128	128	128	NB	4	4	4	16	NB	16	4	4	>16	NB	>16	16	>16	0.5	R	0.25	0.12	0.25	1	R	0.5	0.5	0.5	1	R	0.5	0.5	0.5		NB	2	2	4	0.38	S	0.38	0.5	0.25
5/18/2018	0315	Candida glabrata	4	SDD	4	4	4	NB	0.25	0.12	0.12	1	NB	0.5	0.25	1	1	NB	0.5	0.5	0.5	16	R	>16	>16	>16	2	R	4	4	4	4	R	2	2	2		NB	0.12	0.12	0.12	0.38	S	0.75	0.5	0.38
5/18/2018	0317	Candida glabrata	32	SDD	32	32	32	NB	1	1	1	1	NB	2	1	2	1	NB	2	1	2	1	R	0.5	1	0.5	0.5	R	0.5	1	0.5	0.25	R	0.25	0.25	0.25		NB	1	0.25	0.5	0.19	S	0.38	0.25	0.25
5/18/2018	0318	Candida glabrata	32	SDD	32	32	32	NB	0.5	0.5	0.5	1	NB	1	0.5	1	1	NB	1	0.5	1	16	R	16	16	0.25	4	R	2	2	1	4	R	2	2	0.5		NB	0.5	0.25	1	0.19	S	0.19	0.125	0.125
5/18/2018	0320	Candida glabrata	4	SDD	4	4	4	NB	0.06	0.06	0.12	1	NB	0.12	1	0.12	1	NB	0.5	0.25	0.5	1	R	0.25	0.5	0.25	0.5	R	0.25	0.25	0.25	0.25	R	0.03	0.06	0.06		NB	0.06	0.06	0.12	0.19	S	0.38	0.5	0.38
5/18/2018	0321	Candida glabrata	64	R	64	32	64	NB	1	1	1	2	NB	1	0.5	1	1	NB	1	0.5	1	4	R	2	1	2	2	R	2	2	2	1	R	0.25	0.25	0.25		NB	0.25	0.12	0.25	0.09	S	0.5	0.25	0.5
5/18/2018	0322	Candida glabrata	8	SDD	4	2	4	NB	0.12	0.06	0.12	0.5	NB	0.12	0.12	0.12	0.5	NB	0.5	0.12	0.25	2	R	2	2	0.5	2	R	2	0.5	2	0.25	R	0.25	0.12	0.25		NB	0.06	0.06	0.06	0.19	S	0.5	0.5	0.5
5/18/2018	0323	Candida glabrata	4	SDD	4	2	2	NB	0.06	0.06	0.06	0.25	NB	0.12	0.06	0.12	0.25	NB	0.25	0.12	0.12	16	R	16	4	8	4	R	2	1	2	4	R	2	1	1		NB	0.06	0.03	0.06	0.19	S	1.5	1	0.75
5/18/2018	0324	Candida glabrata	8	SDD	4	2	4	NB	0.06	0.06	0.06	0.5	NB	0.12	0.12	0.12	0.5	NB	0.25	0.12	0.25	16	R	0.25	0.12	0.25	4	R	1	1	0.25	2	R	0.12	0.25	0.12		NB	0.12	0.06	0.12	0.25	S	1	1.5/2	0.75
5/18/2018	0325	Candida glabrata	128	R	256	128	256	NB	8	8	8	8	NB	4	16	4	16	NB	>16	>16	>16	>16	R	>16	16	0.5	4	R	2	2	2	4	R	0.5	1	0.5		NB	4	2	4	0.38	S			
5/18/2018	0327	Candida glabrata	16	SDD	2	2	2	NB	0.06	0.06	0.06	1	NB	0.12	0.12	0.06	1	NB	0.25	0.25	0.12	0.125	S	0.015	0.015	0.015	0.125	S	0.03	0.03	0.015	0.015	S	0.015	0.015	0.015		NB	0.06	0.06	0.06	0.25	S			
5/22/2018	1132	Candida krusei	.	NB	32	32		S	0.25	0.25		0.25	NB	0.25	0.25		0.25	NB	0.5	0.5		.		0.12	0.12		0.12	S	0.03	0.06		0.12	S	0.12	0.12			NB	0.25	0.25		1	R			
5/22/2018	0397	Candida krusei	64	NB	128	128		SDD	1	1		1	NB	0.5	0.5		1	NB	1	0.5		0.125	S	0.25	0.25		0.03	S	0.06	0.12		0.125	S	0.25	0.25			NB	0.25	0.25		.	.			
6/5/2018	0314	Candida glabrata	64	R	128		128	4	NB	4		4	16	NB	4		4	>16	NB	>16		>16	0.5	R	0.25		0.5	1	R	1		1	1	R	1		1		NB	4		4	0.38	S	0.38	0.38	
6/5/2018	0315	Candida glabrata	4	SDD	8		8	0.25	NB	0.12		0.12	1	NB	0.5		1	1	NB	0.5		1	16	R	>16		>16	2	R	4		2	4	R	4		4		NB	0.12		0.25	0.38	S	0.5	1	
6/5/2018	0317	Candida glabrata	32	SDD	32		32	0.5	NB	1		1	1	NB	1		1	1	NB	1		1	1	R	0.5		0.5	0.5	R	0.5		0.5	0.25	R	0.25		0.25		NB	0.5		0.5	0.19	S	0.38	0.5	
6/5/2018	0318	Candida glabrata	32	SDD	64		64	1	NB	1		1	1	NB	2		2	1	NB	2		2	16	R	16		>16	4	R	2		2	4	R	1		1		NB	1		1	0.19	S	0.25	0.38	
6/5/2018	0320	Candida glabrata	4	SDD	8		8	0.12	NB	0.12		0.12	1	NB	0.25		0.25	1	NB	0.5		0.5	1	R	1		4	0.5	R	0.5		0.5	0.25	R	0.06		0.06		NB	0.12		0.12	0.19	S	0.19	0.25	
					DP	AS	AB				DP	AS	AB				DP	AS	AB				DP	AS	AB				DP	AS	AB				DP	AS	AB				DP	AS	AB				DP	AS	AB			
6/19/2018	0321	Candida glabrata	64	R	64	64	64	2	NB	1	1	1	2	NB	1	1	1	1	NB	0.5	0.5	0.5	4	R	4	4	4	2	R	2	2	2	1	R	1	1	1		NB	0.25	0.25	0.25	0.09	S	0.047	0.047	0.125
6/19/2018	0322	Candida glabrata	8	SDD	4	4	4	0.12	NB	0.12	0.12	0.12	0.5	NB	0.12	0.12	0.12	0.5	NB	0.25	0.25	0.25	2	R	>16	>16	>16	2	R	1	1	1	0.25	R	0.5	0.5	0.25		NB	0.06	0.06	0.06	0.19	S	0.25	0.19	0.25
6/19/2018	0323	Candida glabrata	4	SDD	8	8	8	0.06	NB	0.12	0.12	0.12	0.25	NB	0.25	0.25	0.25	0.25	NB	0.25	0.25	0.25	16	R	8	16	8	4	R	2	2	2	4	R	2	2	1		NB	0.06	0.06	0.06	0.19	S	0.5	0.25	1.5
6/19/2018	0324	Candida glabrata	8	SDD	8	8	8	0.25	NB	0.12	0.12	0.12	0.5	NB	0.25	0.25	0.25	0.5	NB	0.5	0.5	0.5	16	R	2	4	2	4	R	2	2	2	2	R	0.12	0.5	0.5		NB	0.12	0.12	0.12	0.25	S	0.38	0.25	0.38
6/19/2018	0325	Candida glabrata	128	R	256	256	256	16	NB	4	4	4	8	NB	4	4	4	16	NB	>16	>16	>16	>16	R	>16	>16	>16	4	R	4	4	4	4	R	2	2	2		NB	4	4	4	0.38	S	0.38	0.38	0.38
					DP	DL	AB				DP	DL	AB				DP	DL	AB				DP	DL	AB				DP	DL	AB				DP	DL	AB				DP	DL	AB				DP	DL	AB			
6/21/2018	327	Candida glabrata	16	SDD	8	8	8	0.25	NB	0.25	0.12	0.12	1	NB	0.5	0.5	0.5	1	NB	0.5	0.5	0.5	0.125	S	0.06	0.06	0.06	0.125	S	0.06	0.06	0.06	0.015	S	0.015	0.015	0.015		NB	0.25	0.12	0.12	0.25	S	0.25		
6/21/2018	922	Candida lusitaniae	2	NB	1	1	1	0.015	NB	0.008	0.008	0.008	0.12	NB	0.03	0.03	0.03	0.25	NB	0.12	0.06	0.12		NB	0.06	0.06	0.06	0.25	NB	0.12	0.12	0.12	0.12	NB	0.06	0.06	0.06		NB	0.015	0.015	0.015	0.5	S	0.047		
6/21/2018	398	Candida lusitaniae	1	NB	0.5	0.5	0.5	0.016	NB	0.008	0.008	0.008	0.5	NB	0.015	0.015	0.015	0.125	NB	0.06	0.06	0.06	0.125	NB	0.12	0.12	0.25	0.125	NB	0.25	0.25	0.25	0.125	NB	0.06	0.06	0.06		NB	0.015	0.015	0.06	0.38	S	0.016		
6/21/2018	339	Candida parapsilosis	32	R	32	32	32	1	R	0.5	0.5	0.5	0.125	S	0.12	0.06	0.12	0.125	S	0.25	0.12	0.25	0.25	S	0.25	0.25	0.25	1	S	2	2	2	1	S	1	1	1		NB	0.12	0.12	0.12	0.047	S	0.125		
6/21/2018	340	Candida parapsilosis	64	R	1	0.5	1	1	R	0.03	0.15	0.03	0.125	S	0.03	0.03	0.06	0.125	S	0.06	0.06	0.12	0.25	S	0.12	0.12	0.12	1	S	2	2	2	0.5	S	1	1	1		NB	0.015	0.015	0.03	0.094	S	0.094		
					DP	AS	DL				DP	AS	DL				DP	AS	DL				DP	AS	DL				DP	AS	DL				DP	AS	DL				DP	AS	DL				DP	AS	DL			
6/28/2018	0336	Candida parapsilosis	32	R	64	128	128	1	R	1	1	2	0.125	S	0.06	0.06	0.06	0.125	S	0.12	0.12	0.5	0.25	S	0.25	0.25	0.25	1	S	1	1	1	1	S	0.5	0.5	1		NB	0.12	0.12	0.25	0.047	S	0.094	0.094	
6/28/2018	0337	Candida parapsilosis	64	R	64	64	128	1	R	1	1	2	0.125	S	0.06	0.06	0.06	0.125	S	0.12	0.12	0.25	0.25	S	0.25	0.25	0.25	1	S	1	1	1	0.5	S	0.5	0.5	1		NB	0.12	0.06	0.12	0.094	S	0.125	0.094	
6/28/2018	0338	Candida parapsilosis	16	R	8	8	16	0.25	I	0.12	0.12	0.25	0.25	S	0.12	0.12	0.12	0.5	S	0.25	0.25	0.5	1	S	0.25	0.25	0.25	1	S	0.5	0.5	1	1	S	1	1	1		NB	0.12	0.12	0.12	0.125	S	0.064	0.047	
6/28/2018	0193	Candida tropicalis	64	R	64	64	128	8	R	8	8	8	1	R	0.5	0.5	0.5	0.5	S	0.5	0.5	1	.	.	1	1	2	1	R	1	0.5	1	1	R	1	1	1		NB	4	4	4	1	R	0.19	0.19	
6/28/2018	0345	Candida tropicalis	>256	R	>256	>256	>256	16	R	>16	>16	>16	>16	R	>16	>16	>16	>16	R	>16	>16	>16	0.06	S	0.03	0.03	0.03	0.06	S	0.03	0.03	0.03	0.06	S	0.03	0.03	0.03		NB	>8	>8	>8	0.38	S	0.25	0.19	
					DP	AV	AB				DP	AV	AB				DP	AV	AB				DP	AV	AB				DP	AV	AB				DP	AV	AB				DP	AV	AB				DP	AV	AB			
7/10/2018	0381	Candida auris	4	S	8	8	8	0.03	NB	0.03	0.03	0.03	0.06	NB	0.015	0.015	0.015	0.125	NB	0.03	0.03	0.03	0.125	S	0.015	0.015	0.015	0.25	S	0.03	0.03	0.03	0.125	S	0.03	0.03	0.03		NB	0.008	0.008	0.015	0.38	S	0.38	0.023	0.032
7/10/2018	0382	Candida auris	16	S	32	>256	>256	0.5	NB	>16	>16	>16	0.5	NB	0.5	1	>16	1	NB	>16	>16	>16	0.5	S	0.5	0.12	1	0.25	S	0.25	0.25	0.5	0.25	S	0.25	0.25	0.12		NB	0.5	0.008	>16	0.38	S	0.25		0.023
7/10/2018	0383	Candida auris	128	R	256	256	>256	4	NB	8	4	4	0.5	NB	0.12	0.12	0.12	0.5	NB	0.5	0.5	0.5	16	R	16	16	0.25	1	S	2	1	1	1	S	0.5	0.25	0.5		NB	0.12	0.25	0.25	0.38	S	0.25	0.023	0.25
7/10/2018	0384	Candida auris	128	R	256	256	>256	1	NB	4	8	4	0.5	NB	0.5	0.12	0.12	1	NB	0.25	0.25	0.5	16	R	16	4	0.06	2	S	1	4	1	2	R	0.25	0.25	0.25		NB	0.06	0.12	0.25	0.5	S	0.25	0.25	0.25
7/10/2018	0385	Candida auris	>256	R	>256	256	>256	16	NB	8	8	8	1	NB	0.25	0.25	0.25	1	NB	0.5	1	0.5	0.5	S	0.25	0.25	0.25	1	S	0.5	0.5	0.5	0.5	S	0.25	0.12	0.25		NB	1	1	1	0.5	S	0.25	0.5	0.5
7/10/2018	0386	Candida auris	>256	R	>256	256	>256	16	NB	8	16	8	0.5	NB	0.25	0.25	0.25	0.5	NB	0.5	0.5	0.5	0.5	S	0.25	0.25	0.12	1	S	0.5	0.5	0.5	0.25	S	0.25	0.25	0.25		NB	1	1	1	0.5	S	0.38		0.064
						AV	AS					AV	AS					AV	AS					AV	AS					AV	AS					AV	AS					AV	AS					AV	AS			
7/13/2018	0387	Candida auris	8	S		8	8	0.6	NB		0.5	0.25	0.25	NB		0.12	0.06	0.5	NB		0.12	0.25	0.25	S		0.12	0.12	0.5	S		0.5	0.5	0.5	S		0.12	0.12		NB		0.06	0.03	0.75	S	0.38 48h	0.125	0.125
7/13/2018	0388	Candida auris	>256	R		>256	>256	2	NB		2	2	0.25	NB		0.12	0.25	0.5	NB		0.5	1	1	S		0.25	0.25	0.5	S		0.25	0.25	0.125	S		0.25	0.12		NB		0.5	1	1.5	S	1 48h	1.5	0.75
7/13/2018	0389	Candida auris	256	R		>256	>256	4	NB		4	4	0.125	NB		0.12	0.12	0.25	NB		0.5	0.5	0.5	S		0.25	0.25	1	S		0.25	1	0.25	S		0.25	0.25		NB		0.25	0.5	4	R	1 48h	2	1
7/13/2018	0390	Candida auris	>256	R		256	>256	8	NB		2	2	0.5	NB		0.06	0.25	1	NB		0.5	0.5	0.5	S		0.06	0.12	1	S		0.12	1	0.25	S		0.12	0.25		NB		0.5	1	4	R	0.5 48h	0.75	0.5
7/13/2018	0335	Candida parapsilosis	16	R		16	16	1	R		0.5	0.5	0.25	S		0.03	0.03	0.5	S		0.12	0.12	0.5	S		0.25	0.25	4	I		2	2	1	S		0.5	1		NB		0.06	0.06	0.19	S	0.38 48h	0.32	0.125
					DP	DL	AB				DP	DL	AB				DP	DL	AB				DP	DL	AB				DP	DL	AB				DP	DL	AB				DP	DL	AB				DP	DL	AB			
8/2/2018	0318	Candida glabrata	32	SDD	64	64	64	1	NB	1	2	1	1	NB	2	2	2	1	NB	2	2	2	16	R	16	>16	>16	4	R	2	4	4	4	R	4	4	4		NB	1	2	2	0.19	S	0.125/0.38		0.125
8/2/2018	0320	Candida glabrata	4	SDD	8	8	8	0.12	NB	0.25	0.5	0.25	1	NB	0.5	0.5	0.5	1	NB	0.5	0.5	0.5	1	R	0.12	14	0.5	0.5	R	0.25	1	0.5	0.25	R	0.06	0.5	0.06		NB	0.12	0.25	0.12	0.19	S			0.5
8/2/2018	0322	Candida glabrata	8	SDD	8	8	8	0.12	NB	0.12	0.12	0.12	0.5	NB	0.25	0.25	0.25	0.5	NB	0.25	0.5	0.25	2	R	2	>16	>16	2	R	1	2	1	0.25	R	0.5	0.5	0.5		NB	0.06	0.12	0.12	0.19	S	0.19/0.25		0.125
8/2/2018	0324	Candida glabrata	8	SDD	8	8	8	0.25	NB	0.12	0.12	0.12	0.5	NB	0.5	0.25	0.5	0.5	NB	0.5	0.5	0.5	16	R	0.25	>16	0.25	4	R	2	4	1	2	R	0.5	2	0.5		NB	0.25	0.25	0.25	0.25	S	0.25/0.5		0.5
8/3/2018	0382	Candida auris	16	S	8	>256	256	0.5	NB	0.03	16	16	0.5	NB	0.015	16	16	1	NB	0.5	>16	16	0.5	S	0.06	2	0.12	0.25	S	0.25	1	0.5	0.25	S	0.12	0.12	0.12		NB	0.008	8	8	0.38	S	0.094/0.25		0.064
8/3/2018	0384	Candida auris	128	R	128	>256	>256	1	NB	2	16	4	0.5	NB	0.015	0.12	0.06	1	NB	0.25	0.25	0.25	16	R	0.12	0.25	0.25	2	S	0.25	4	0.5	2	R	0.06	0.5	0.12		NB	0.06	0.25	0.06	0.5	S	0.25/0.5		0.19
					DP	AS	AB				DP	AS	AB				DP	AS	AB				DP	AS	AB				DP	AS	AB				DP	AS	AB				DP	AS	AB				DP	AS	AB			
8/7/2018	0327	Candida glabrata	16	SDD	4	4	4	0.25	NB	0.12	0.12	0.12	1	NB	0.5	0.25	0.5	1	NB	0.5	0.5	0.5	0.125	S	0.06	0.06	0.06	0.125	S	0.06	0.06	0.03	0.015	S	0.008	0.008	0.015		NB	0.12	0.06	0.12	0.25	S			
8/7/2018	0335	Candida parapsilosis	16	R	16	16	16	1	R	0.25	0.25	0.5	0.25	S	0.06	0.03	0.06	0.5	S	0.12	0.12	0.12	0.5	S	0.25	0.25	0.25	4	I	2	2	2	1	S	1	1	1		NB	0.03	0.03	0.06	0.19	S			
8/7/2018	0338	Candida parapsilosis	16	R	16	16	16	0.25	I	0.25	0.25	0.12	0.25	S	0.25	0.12	0.12	0.5	S	0.5	0.25	0.5	1	S	0.25	0.25	0.5	1	S	1	2	1	1	S	1	1	1		NB	0.12	0.12	0.12	0.125	S			
8/7/2018	0390	Candida auris	>256	R	>256	256	>256	8	NB	2	1	0.5	0.5	NB	0.12	0.06	0.06	1	NB	0.5	0.5	0.25	0.5	S	0.06	0.03	0.06	1	NB	0.5	0.25	0.12	0.25	S	0.12	0.12	0.12		NB	0.5	0.5	0.12	4	R	0.5	0.75	0.75
8/7/2018	0193	Candida tropicalis	64	R	128	64	64	8	R	8	8	8	1	R	0.5	0.5	0.25	0.5	S	0.5	0.5	0.5	.	.	2	2	1	1	R	1	1	1	1	R	1	1	1		NB	4	4	4	1	S			
8/7/2018	0389	Candida auris	256	R				4	NB				0.125	NB				0.25	NB				0.5	S				1	S				0.25	S					NB				4	R	1.5/2	2	1.5
8/7/2018	0390	Candida auris	>256	R				8	NB				0.5	NB				1	NB				0.5	S				1	S				0.25	S					NB				4	R	0.5/0.75	0.75	0.75
8/7/2018	1132	Candida krusei	.	NB				0.25	S				0.25	NB				0.25	NB				.					0.12	S				0.12	S					NB				1	R	0.125/0.5	0.5	0.25

Was it us?- Repeat testing

- Repeated testing
  - Fresh isolates from the freezer
  - Confirmed at least 2 passes from the freezer and 24 hours old
  - Additional people doing set-up and reading
- No change in results!

C. auris

Caspofungin

	CDC		DP	AV	AB
1	0.03	NB	0.03	0.03	0.03
2	**0.5**	NB	>16	>16	>16
3	4	NB	8	4	4
4	1	NB	4	8	4
5	16	NB	8	8	8

Was it Us?

- Double checked protocol, discussed with CDC
- Ensured viability and CFU based on turbi
- Tried manual vs automatic set-up (Sensit
- Tried plate films vs lids
- Ordered fresh QC isolates
- Compared plate lots
- Compared results between readers
- Asked for reader training from CDC
- Discussed issues with other labs bringing up this testing

Clicker Question

What would you do next?

A. Pass the Validation
B. Give up
C. More repeat testing
D. Phone a friend

Was it the Isolates? Tie Breaker Testing

- Consulted with CDC,
  - They agreed to test the most discrepant strains
  - We sent our current strains (they did not pull from the AR Bank)

- Everything re-test by CDC matched our results!!!!

- Requested CDC test the remaining discrepants
  - They did not have the capacity to help with this

Tie Breaker– 3rd Lab

Minnesota State Laboratory also runs this test and agreed to test our isolates

	Fluconazole					
	CDC		WSLH		MN	
	MIC	INT	MIC	INT	MIC	INT
Candida lusitaniae	1	NB	0.5	NB	0.5	NB
Candida glabrata	32	SDD	64	R	64	R
Candida glabrata	4	SDD	8	SDD	4	SDD
Candida glabrata	8	SDD	8	SDD	4	SDD
Candida parapsilosis	16	R	16	R	16	R

NB=No Breakpoints, SDD=Susceptible dose dependent, R=Resistant

Candida AST Accuracy Data

			Fluconazole				Voriconazole				Posaconazole				Itraconazole				Caspofungin				Anidulafungin				Micafungin				Isavuconazole				Amphotericin B			
			CDC		WSLH		CDC		WSLH		CDC		WSLH		CDC		WSLH		CDC		WSLH		CDC		WSLH		CDC		WSLH		CDC		WSLH		CDC		WSLH	
			MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT
1	0381	Candida auris	4	S	8	S	0.03	NB	0.03	NB	0.06	NB	0.02	NB	0.125	NB	0.03	NB	0.13	S	0.02	S	0.25	S	0.5	S	0.125	S	0.03	S	0.125	NB	0.25	NB	0.38	S	0.4	S
2	0383	Candida auris	128	R	256	R	4	NB	8	NB	0.5	NB	0.12	NB	0.5	NB	0.5	NB	16	R	16	R	1	S	2	S	1	S	0.25	S	0.5	NB	1	NB	0.38	S	0.5	S
3	0384	Candida auris	128	R	256	R	1	NB	4	NB	0.5	NB	0.12	NB	1	NB	0.25	NB	**0.5**	S	0.12	S	2	S	1	S	2	S	0.25	S	0.25	NB	0.12	NB	0.5	S	0.5	S
4	0385	Candida auris	>256	R	>256	R	16	NB	8	NB	1	NB	0.25	NB	1	NB	0.5	NB	0.5	S	0.25	S	1	S	0.5	S	0.5	S	0.25	S	1	NB	1	NB	0.5	S	0.8	S
5	0386	Candida auris	>256	R	>256	R	16	NB	8	NB	0.5	NB	0.25	NB	0.5	NB	0.5	NB	0.5	S	0.25	S	1	S	0.5	S	0.25	S	0.25	S	-	NB	0.06	NB	0.5	S	0.4	S
6	0387	Candida auris	8	S	8	S	0.6	NB	0.5	NB	0.25	NB	0.12	NB	0.5	NB	0.12	NB	0.25	S	0.12	S	0.5	S	0.5	S	0.5	S	0.12	S	0.5	NB	0.5	NB	0.75	S	0.1	S
7	0388	Candida auris	>256	R	>256	R	2	NB	2	NB	0.25	NB	0.12	NB	0.5	NB	0.5	NB	1	S	0.25	S	0.5	S	0.25	S	0.125	S	0.25	S	0.5	NB	0.5	NB	1.5	S	1.5	S
8	0389	Candida auris	256	R	>256	R	4	NB	4	NB	0.13	NB	0.12	NB	0.25	NB	0.5	NB	0.5	S	0.25	S	1	S	0.25	S	0.25	S	0.25	S	0.25	NB	0.25	NB	4	R	2	R
9	0390	Candida auris	>256	R	256	R	8	NB	2	NB	0.5	NB	0.12	NB	1	NB	0.5	NB	0.5	S	0.06	S	1	S	0.25	S	0.25	S	0.12	S	0.016	NB	0.008	NB	**0.75**	S	0.8	S
10	0314	Candida glabrata	64	R	128	R	4	NB	4	NB	16	NB	4	NB	>16	NB	>16	NB	0.5	R	0.5	R	1	R	1	R	1	R	1	R	-	NB	4	NB	0.38	S	0.4	S
11	0315	Candida glabrata	4	SDD	8	SDD	0.25	NB	0.12	NB	1	NB	0.5	NB	1	NB	0.5	NB	16	R	>16	R	2	R	4	R	4	R	4	R	-	NB	1	NB	0.38	S	0.5	S
12	0317	Candida glabrata	32	SDD	32	SDD	0.5	NB	1	NB	1	NB	1	NB	1	NB	1	NB	1	R	0.5	R	0.5	R	0.5	R	0.25	R	0.25	R	-	NB	0.5	NB	0.19	S	0.4	S
13	0318	Candida glabrata	32	SDD	64	R	1	NB	1	NB	1	NB	2	NB	1	NB	2	NB	16	R	16	R	4	R	2	R	4	R	1	R	-	NB	0.12	NB	0.19	S	0.3	S
14	0319	Candida glabrata	4	SDD	8	SDD	0.12	NB	0.12	NB	0.25	NB	0.25	NB	0.5	NB	0.5	NB	1	R	1	R	0.5	R	2	R	2	R	0.5	R	-	NB	0.25	NB	0.13	S	0.38	S
15	0320	Candida glabrata	4	SDD	8	SDD	0.12	NB	0.12	NB	1	NB	0.25	NB	1	NB	0.5	NB	1	R	1	R	0.5	R	0.5	R	0.25	R	0.06	S	-	NB	0.06	NB	0.19	S	0.2	S
16	0321	Candida glabrata	64	R	64	R	2	NB	1	NB	2	NB	1	NB	1	NB	0.5	NB	4	R	4	R	2	R	2	R	1	R	1	R	-	NB	0.25	NB	0.09	S	0	S
17	0322	Candida glabrata	8	SDD	4	SDD	0.12	NB	0.12	NB	0.5	NB	0.12	NB	0.5	NB	0.25	NB	2	R	2	R	2	R	1	R	0.25	R	0.5	R	-	NB	0.06	NB	0.19	S	0.2	S
18	0323	Candida glabrata	4	SDD	8	SDD	0.06	NB	0.12	NB	0.25	NB	0.25	NB	0.25	NB	0.25	NB	16	R	8	R	4	R	2	R	4	R	2	R	-	NB	0.06	NB	0.19	S	0.3	S
19	0324	Candida glabrata	8	SDD	8	SDD	0.25	NB	0.12	NB	0.5	NB	0.25	NB	0.5	NB	0.5	NB	16	R	2	R	4	R	2	R	2	R	0.5	R	-	NB	0.25	NB	0.25	S	0.3	S
20	0325	Candida glabrata	128	R	256	R	16	NB	4	NB	8	NB	4	NB	16	NB	>16	NB	>16	R	>16	R	4	R	4	R	4	R	2	R	-	NB	4	NB	0.38	S	0.4	S
21	0327	Candida glabrata	16	SDD	8	SDD	0.25	NB	0.25	NB	1	NB	0.5	NB	1	NB	0.5	NB	0.13	S	0.06	S	0.125	S	0.06	S	0.015	S	0.015	S	-	NB	0.12	NB	0.25	S	0.3	S
22	0330	Candida glabrata	8	SDD	8	SDD	0.25	NB	0.25	NB	1	NB	0.5	NB	1	NB	0.5	NB	0.06	S	0.06	S	0.03	S	0.06	S	0.015	S	0.015	S	-	NB	0.25	NB	-	-	-	-
23	0331	Candida glabrata	64	R	64	R	1	NB	1	NB	2	NB	2	NB	1	NB	4	NB	0.06	S	0.06	S	0.03	S	0.06	S	0.015	S	0.015	S	-	NB	1	NB	-	-	-	-
24	0332	Candida glabrata	128	R	64	R	4	NB	2	NB	2	NB	2	NB	1	NB	4	NB	0.06	S	0.12	S	0.06	S	0.06	S	0.015	S	0.015	S	-	NB	2	NB	-	-	-	-
26	1132	Candida krusei	-	NB	32	NB	0.25	S	0.25	S	0.25	NB	0.25	NB	0.25	NB	0.5	NB	-	-	-	-	0.12	S	0.03	S	0.12	S	0.12	S	-	NB	0.25	NB	1	S	0.5	S
27	0397	Candida krusei	64	NB	128	NB	1	SDD	1	SDD	1	NB	0.5	NB	1	NB	1	NB	0.13	S	0.25	S	0.03	S	0.06	S	0.125	S	0.25	S	-	NB	0.25	NB	-	-	-	-
28	922	Candida lusitaniae	2	NB	1	NB	0.02	NB	0.01	NB	0.12	NB	0.03	NB	0.25	NB	0.12	NB	-	NB	-	NB	0.25	NB	0.12	NB	0.12	NB	0.06	NB	-	NB	0.015	NB	0.5	*	0.1	*
29	0398	Candida lusitaniae	1	NB	0.5	NB	0.02	NB	0.01	NB	0.5	NB	0.02	NB	0.125	NB	0.06	NB	0.13	NB	0.12	NB	0.125	NB	0.25	NB	0.125	NB	0.06	NB	-	NB	0.015	NB	0.38	*	0.1	*
30	0335	Candida parapsilosis	16	R	16	R	1	R	0.5	I	0.25	NB	0.06	NB	0.5	NB	0.12	NB	0.5	S	0.25	S	4	I	2	S	1	S	0.5	S	-	NB	0.06	NB	0.19	S	0.3	S
31	0336	Candida parapsilosis	32	R	64	R	1	R	1	R	0.13	NB	0.06	NB	0.125	NB	0.12	NB	0.25	S	0.25	S	1	S	1	S	1	S	0.5	S	-	NB	0.12	NB	0.05	S	0.1	S
32	0337	Candida parapsilosis	64	R	64	R	1	R	1	R	0.13	NB	0.06	NB	0.125	NB	0.12	NB	0.25	S	0.25	S	1	S	1	S	0.5	S	0.5	S	-	NB	0.12	NB	0.09	S	0.1	S
33	0338	Candida parapsilosis	16	R	8	R	0.25	I	0.25	I	0.25	NB	0.12	NB	0.5	NB	0.25	NB	1	S	0.25	S	1	S	0.5	S	1	S	1	S	-	NB	0.12	NB	0.13	S	0.1	S
34	0339	Candida parapsilosis	32	R	32	R	0.5	I	0.5	I	0.25	NB	0.12	NB	0.25	NB	0.25	NB	0.25	S	0.25	S	1	S	2	S	1	S	1	S	-	NB	0.12	NB	0.05	S	0.1	S
35	0340	Candida parapsilosis	0.05	S	1	S	0.02	S	0.03	S	0.13	NB	0.03	NB	0.125	NB	0.06	NB	0.25	S	0.12	S	2	S	2	S	0.5	S	1	S	-	NB	0.015	NB	0.03	S	0.1	S
36	0193	Candida tropicalis	64	R	64	R	8	R	8	R	1	NB	0.5	NB	0.5	NB	0.5	NB	-	-	-	-	1	R	1	R	1	R	1	R	-	NB	4	NB	1	S	0.2	S
37	0345	Candida tropicalis	>256	R	>256	R	16	R	>16	R	>16	NB	>16	NB	>16	NB	>16	NB	0.06	S	0.03	S	0.06	S	0.03	S	0.06	S	0.03	S	-	NB	>8	NB	0.38	S	0.3	S
		% minor errors				3.1				10.0				N/A				N/A				0.0				2.9				0.0				N/A				0.0	<10% of isolates
		% major errors				0.0				0.0				N/A				N/A				0.0				0.0				0.0				N/A				0.0	<3% of the susceptible isolates
		% Very major errors				0.0				0.0				N/A				N/A				0.0				0.0				9.1				N/A				0	<3% of the resistant isolates
		Total % catagorocal agreement in				**97.2**				**90.0**				**N/A**				**N/A**				**100**				**100**				**97.1**				**N/A**				**100**	>90% agree and <3% very major errors
		Total % essential agreement out			**0**				**0**				**2.8**				**0**				**2.9**				**0.0**				**0**				**0.0**				**0.0**	<3% of tests

Key

minor error	S to I, I to S, and I to R (SDD=I)
major error	S to R
Very major error	R to S
>2 doubling dilutions	Essential agreement
S	Susceptible
SDD	Susceptible dose dependant
I	Intermediate
R	Resistant

NB	No Breakpoints
	CLSI breakpoints
	EUCAST breakpoints
	CDC breakpoints
	Pass
	Fail

Total validation Catagorical agreement	**97.7**	**0.58**
Total validation Essential agreement		**0.70**

>90% agree and <3% very major errors

<3% of total tests

Things Can Go Wrong

- Difficult to read results
- Bad QC strains
- Bad lot of plates
- Plates thawing in transport and spilling contents
- Dilution broths with un-equal volume
- One drug in the plate degraded much faster than the shelf life
- Inaccurate data/shift in results from gold standard lab

Key Takeaways

- Double check protocols
- Repeat Testing
- New or fresh isolates
  - Loss of resistance in passage
- Ask original lab to re-test or check their data
- Tie breaker lab
- Adding more specimens (exponential slide)
- Give up
  - Bad test
  - Alternate test methods

Resources page

- CLSI documents:
  - CLSI M52: Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems. 1st Ed. 2015. (https://clsi.org/standards/products/microbiology/documents/m52/)
- CLSI validation guides and webinars:
  - CLSI Breakpoint Implementation Toolkit (BIT): https://clsi.org/meetings/ast/breakpoints-in-use-toolkit/
- AR bank: https://wwwn.cdc.gov/ARIsolateBank/

Quality Control Organism Frequency, Maintenance, and Troubleshooting

Macy Wood, PhD, D(ABMM), Assistant Professor, Department of Pathology[1],
Associate Director, Clinical Microbiology Laboratory[2]
Caitlin Cahak, MLS (ASCP)[CM] Supervisor[2]
Will Laudon, MB (ASCP), Technical Specialist[2]

[1] Medical College of Wisconsin, Milwaukee, WI
[2] Wisconsin Diagnostic Laboratories, Milwaukee, WI

QUALITY CONTROL (QC) ORGANISM

- Overview
  - QC frequency and quality control plans
  - Staff training and documentation
  - QC strain maintenance
  - QC failure troubleshooting and lessons learned

QUALITY CONTROL (QC) STRAINS

- Ensure
  - Precision and accuracy of results
  - Performance of reagents and equipment
  - Performance of staff who carry out testing and report results
- Patient impact
  - Quality/accuracy of patient results
  - Time to results
  - Ability to de-escalate antibiotics
  - QC failure directly impacts care

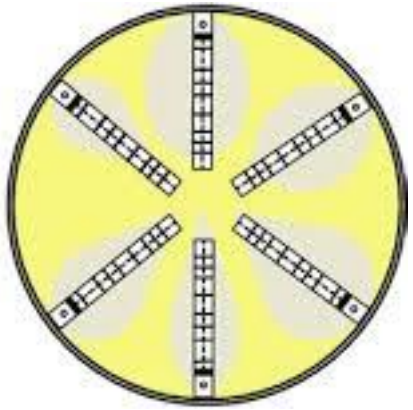
ANTIMICROBIAL SUSCEPTIBILITY TESTING (AST) QC

- QC strain recommendations and QC ranges
  - CLSI M100, M45
    - Disk diffusion or broth microdilution (BMD)

CLSI M100		**Information**
Tables 2A – 2J		Routine QC; testing conditions, breakpoints
Appendix C		QC strains for AST; organism characteristics (resistance mechanisms)
Appendix I **(35th Ed)		**Selection of Quality Control Strains and Quality Control Testing Frequency**
Table 4C		Reference Guide to QC Frequency AST Systems
Disk Diffusion	**MIC (BMD)**	
Table 4A-1 & 2	Table 5A-1 & 2	QC Ranges for Nonfastidious Organisms and Antimicrobial Agents & β-Lactam Combination Agents
Table 4B	Table 5B	QC Ranges for Fastidious Organisms
Table 4D	Table 5G	Troubleshooting Guide for out of range QC

AST QC

- QC strain recommendations/ranges
  - Manufacturer instructions
    - Gradient diffusion strips
    - Commercial/Automated AST



QC LAB REPORT

03/25/2025 05:47:43PM Page 1/1

Wisconsin Diagnostic Laboratory
9200 W. Wisconsin Ave.
Milwaukee, WI 53226

EpiCenter Version: V7.45A / V7.31A
Phoenix Instrument Version: 2.80.0.0

Panel Lot #:	5014161	Expiration Date: 01/09/2026
QC Accession #:		
Sequence Number:	502926844493	
Panel Type:	NMIC-306	Location: 2/B03
Status:	Complete	Tech ID: KB

Isolate Number: 0

Test Strain: 700603 Klebsiella pneumoniae

Start Date/Time:	03/21/2025 03:08:00PM	Test End Date/Time: 03/22/2025 07:06:45AM
ID Broth Lot #:	4354635	Expiration Date: 12/19/2025
Phoenix AP ID Broth Lot #:	4317096	Expiration Date: 11/11/2025
AST Broth Lot #:	4331927	Expiration Date: 11/20/2025
Emerge AST 4.5 mL Broth Lot #:	4284363	Expiration Date: 10/01/2025
Indicator Lot #:	4325005	Expiration Date: 11/27/2025
Organism:	Unspecified	
QC Status:	**Pass**	

AST Results

Antimicrobial	Instrument MIC	Expected MIC	Pass/Fail
Amikacin	<=8		
Amoxicillin-Clavulanate	8/4		
Ampicillin	>16		
Ampicillin-Sulbactam	16/8		
Aztreonam	>16		
Cefazolin	>16		
Cefepime	<=1		
Cefoxitin	>16		
Ceftaroline	>1		
Ceftazidime	16		
Ceftazidime-Avibactam	0.5/4	<=2/4	Pass
Ceftolozane-Tazobactam	<=1/4	0.5/4-2/4	Pass
Ceftriaxone	16		
Cefuroxime	>16		
Ciprofloxacin	1		
Ertapenem	<=0.25		
Gentamicin	8		
Levofloxacin	1		
Meropenem	<=0.5		
Meropenem-Vaborbactam	<=2/8	<=0.13/8	Pass
Minocycline	>8		
Moxifloxacin	2		

FREQUENCY OF QC

- Each day of testing per CMS and CAP (MIC.21910)
  - "Daily", Time of Testing (TOT)
- Reduced AST QC Frequency
  - Weekly, **Monthly**, etc.
  - <u>Performance Criteria</u>
    - A) 20- or 30- day plan
    - B) 15 replicate plan
  - <u>Individualized Quality Control Plan (IQCP)</u>
    - Approved by lab director

AST Method/Topic	CLSI resource for QC frequency
Disk diffusion	M02
Broth & Agar dilution	M07
QC Commercial ID systems	M50
Commercial ID/AST verification	M52
MIC guide to QC frequency	M100 – Table 5F
QC strain selection/frequency	M100 – Appendix I

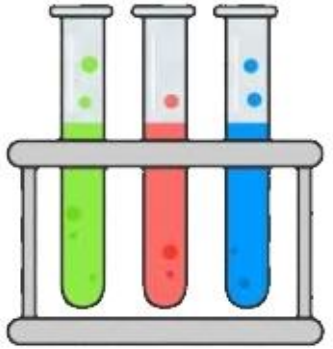
REDUCING QC FREQUENCY

- A) 20- or 30- day plan
  - QC strain/s tested for 20- consecutive test days
    - Single replicate of strain/s
  - Document results

Acceptable ***each antibiotic/QC strain combination**	**Unacceptable**
0-1 value out of range (20 test days)	Failure to meet criteria
If 2-3 errors, continue 10 more days of testing	Continue daily QC testing
<=3 out of range of 30 test days	Corrective action/investigation

REDUCING QC FREQUENCY

- B) 15-replicate plan (3- x 5 days)
  - Three replicates QC strain/s tested for five consecutive test days
    - 3 separate inoculum preparations
    - Different laboratory staff
  - Document results



Acceptable ***each antibiotic/QC strain combination**	**Unacceptable**
0-1 value out of range (15 replicates)	Failure to meet criteria
If 2-3 failures, perform additional 15 replicate	Continue daily QC testing
<=3 out of range of 30 replicates	Corrective action/investigation

INDIVIDUALIZED QUALITY CONTROL PLAN (IQCP)

- Susceptibility Test QC Frequency (MIC.21910)
  - IQCP required *if* performing QC less than indicated by CMS/CAP
    - Cannot be less than manufacturers instructions
  - Requires internal control
    - Exception: **AST systems**, microbiology media/reagents
- Components
  - Risk Assessment (COM.50300)
  - Quality Control Plan (COM.50500)
  - Quality Assessment (COM.50600)

IQCP Resources
Cap.org, E-LAB Solutions Suite, IQCP toolbox
CAP checklist (MIC and COM)
CLSI EP23
Asm.org

IQCP

1) Risk Assessment
- Evaluate potential failures and sources of error/s in your testing process
  - Data review 1-2 years
- 5 components (minimum**):
  - Specimen
    - Labeling, organism viability, isolate age, purity, inoculum suspension
  - Test system
    - Manufacturer package insert, preventative maintenance, software/reporting rules, LIS
  - Reagent
    - Expiration date, preparation, storage, QC recommendations
  - Environment
    - Temperature around test system, reagent storage (Refrigerator/Freezers)
  - Testing personnel
    - Training, competency, PT

IQCP

2) Quality control plan (QCP)

- Processes in place to reduce failure/errors and ensure accuracy of results
- *Possible* Components
  - External controls
    - Daily/Weekly QC documented/reviewed
    - Completed Problem logs reviewed
  - Calibration
    - Instrument, nephelometer documented/reviewed
  - Maintenance
    - Performed at intervals per vendor recommendations
  - Proficiency testing (PT)
    - Documented/reviewed; unsatisfactory results investigated
  - Training and competency assessment
    - Initial, 6 mo, and annually, documented/reviewed; re-training as needed
  - Daily microbiology report review
    - Review AST results, mixed organisms

IQCP

3) Quality Assessment (QA)

- Continuous process of monitoring the QCP effectiveness

  - Practices, processes, and resources to consider for monitoring effectiveness may include:
    - QC reviews
    - Corrected report review
    - Problem log review
    - Temperature review
    - Calibration documentation review
    - PT performance reviews
    - Provider complaint reports

IQCP

- Organization
  - Table format
- Record retention
  - Original + Data
    - Life of system/IQCP use
  - QA review
    - At least every 2 years

Risk Assessment		
Risk Assessment Components	**Sources of Error**	**Error Mitigation**
	Gather information, from the manufacturer's instructions and other resources, on how we should be performing the testing process.	*Indicate how to reduce possible error sources.* • *Internal controls* • *Actions taken by laboratory* • *Safeguards in the test system or*
Specimen	Mislabeled or improperly labeled specimens.	Personnel are trained to properly identify and label patient specimens according to the Labeling of Specimens, DLO-PRE-001 policy. Evaluate reports related to mislabeled specimens and follow corrective action guidelines listed in Rules of Employee Conduct & Progressive Corrective Action policy.
	Specimen received beyond stable period as defined in the applicable policy (see Quality Control Plan)	Testing personnel are trained to verify the collection time and to reject specimens outside of the accepted stability.

TYPES OF QC ERRORS

- Random error
  - QC ranges established using >=95% of results from QC strains
  - Test performed correctly and results still out of range
    - Resolved by repeat testing
- Identifiable error
  - Human error, wrong isolate, mixed organism, mis-read or reported results, etc.
- System error
  - Unknown source, recurring error: inoculum, test system, organism, or reagent, etc.

RANDOM OR IDENTIFIABLE ERROR

Weekly QC
Out of range value/s for weekly QC strain/s **each antibiotic/organism combination*
Action: Repeat QC (same day or with new isolate)
If passed, then still on weekly QC (IQCP) Document results

- Random
  - Occasional out of QC range
- Identifiable
  - QC strain purity plate is mixed
  - Non-viable organism
  - Incorrect QC strain set up
  - Incorrect reagents used
  - Wrong incubation temperature

SYSTEM ERROR

Repeated Weekly QC
2 out of range values per QC strain Repeat failure (x2)
Action: Stop patient testing, suppress antibiotic/s
Begin daily QC testing Corrective action/investigation

- Repeated failure
- Unknown source/issue

SYSTEM ERROR ACTIONS

- Stop patient testing, suppress antibiotic/s
  - *Note: All patient results reported after the last passed QC are at risk*
- Start daily QC testing
  - Report patient results if/when daily QC passes
- Evaluate backup methods
  - Extended downtime?
  - Disk or gradient diffusion, send out testing
- Discuss with clinical colleagues
  - Infectious disease physicians, antimicrobial stewardship, pharmacy, etc.

HOW DO I GET BACK ON IQCP?

ICQP action

- Investigate
  - Identify root cause/troubleshooting
- Obtain fresh isolate, reagents, etc.
- Re-establish reproducibility
  - Begin 20- day QC or 15 replicate (3- x 5 day)
  - Document results

EXAMPLE - FAILED AST QC

- Day 1: *P. aeruginosa* ATCC 27853 QC failed two antibiotics (GNR panel)
  - Ceftazidime and piperacillin/tazobactam (P/T)
  - Test down for all GNRs, not just for *P. aeruginosa*
- Interim action
  - Suppress ceftazidime and P/T
- Backup methods
  - Disk/gradient diffusion
    - Enterobacterales - *E. coli* QC - set up
    - *P. aeruginosa* – *P. aeruginosa* QC- set up

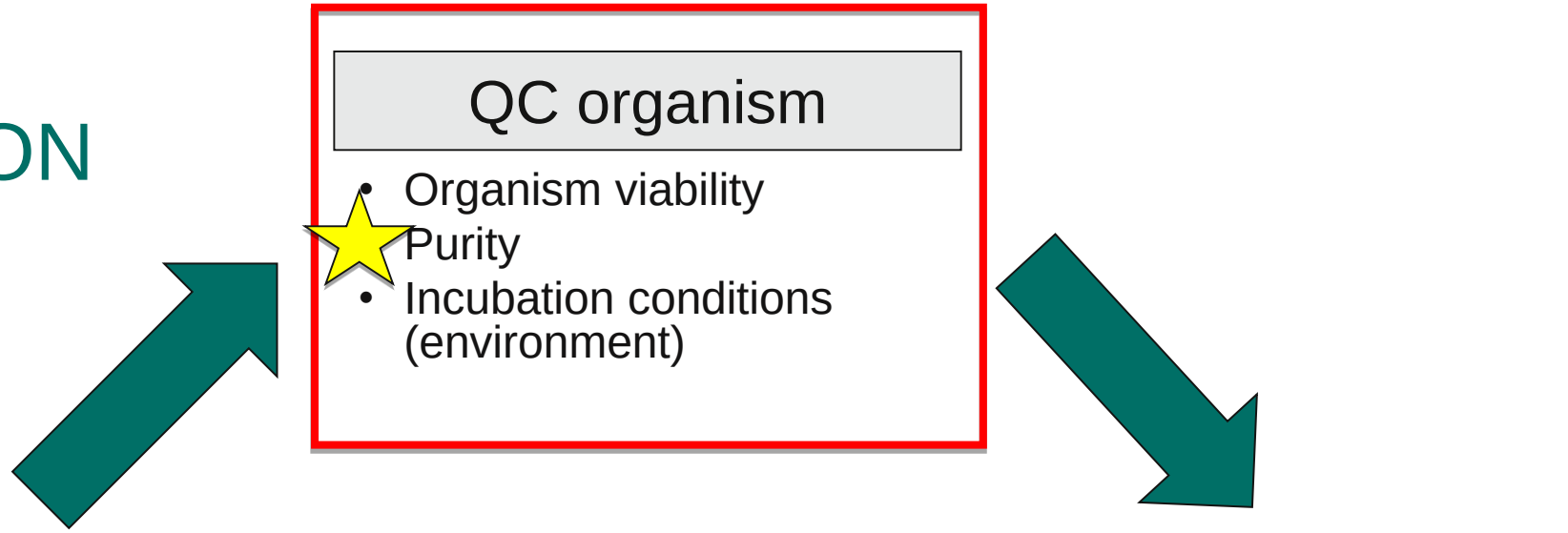
EXAMPLE - FAILED AST QC

- Day 2: *P. aeruginosa* ATCC 27853 QC failed two antibiotics
  - Ceftazidime and piperacillin/tazobactam (P/T)
  - Test down for all GNRs, not just for *P. aeruginosa*
- Interim action
  - Suppress ceftazidime and P/T
- Backup methods
  - Disk/gradient diffusion
    - Enterobacterales - *E. coli* QC - **PASSED**
    - *P. aeruginosa* – *P. aeruginosa* QC- **FAILED**

EXAMPLE - FAILED AST QC

- Clinical communication
  - Ceftazidime
    - Not on formulary, not used, result not needed
  - Piperacillin/tazobactam (P/T)
    - Enterobacterales – not used as frequently
      - High volume
      - Test P/T upon request via KB
    - *P. aeruginosa* – P/T routinely used
      - Test P/T after QC passes
      - Saved isolates to test and report results

QC FAILURE INVESTIGATION



Reagent issues (Media or Antibiotic)

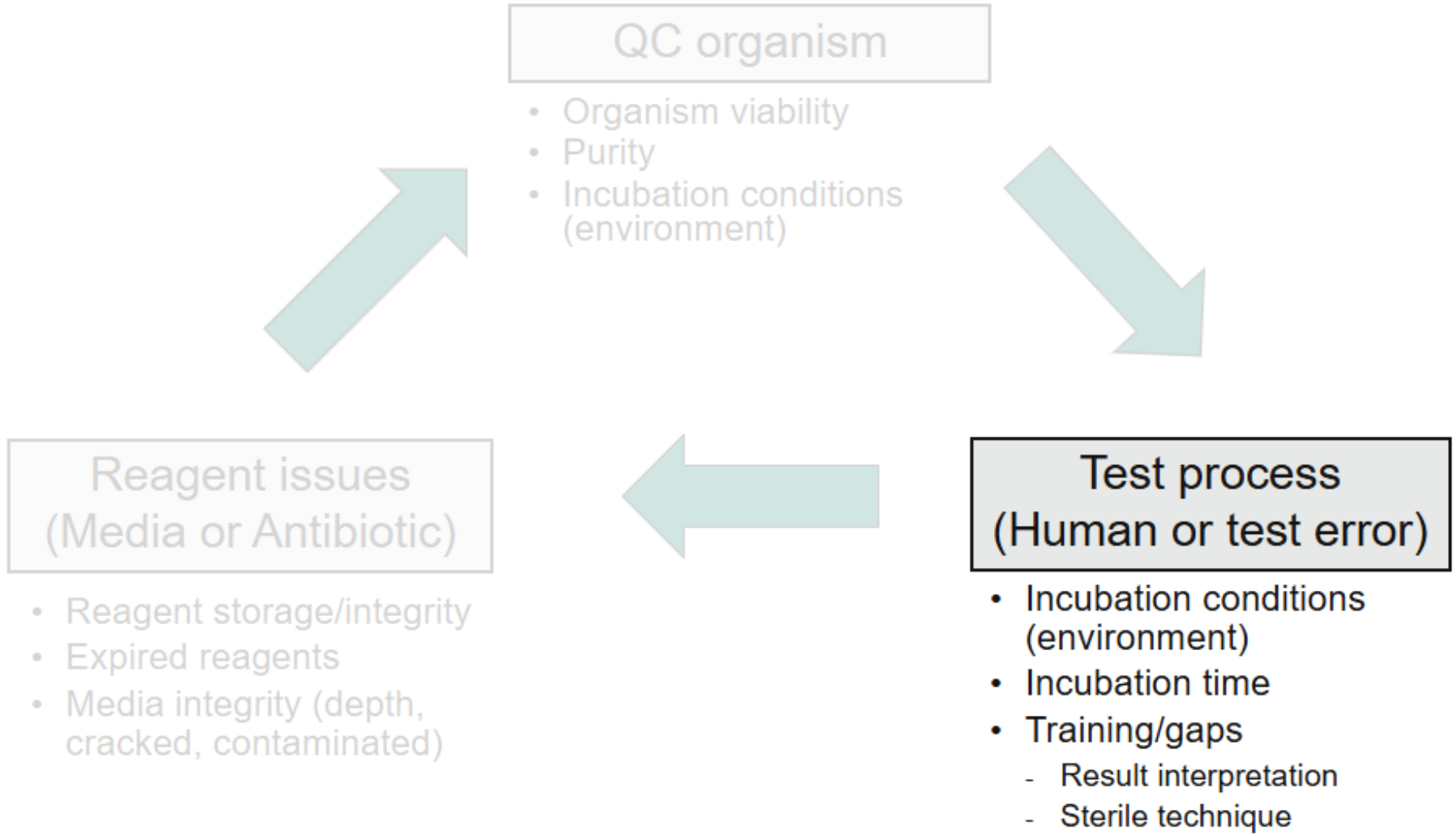
- Reagent storage/integrity
- Expired reagents
- Media integrity (depth, cracked, contaminated)

Test process (Human or test error)

- Incubation conditions (environment)
- Incubation time
- Training/gaps
  - Result interpretation
  - Sterile technique

TROUBLESHOOTING & RCA

- Training and education gaps
- Organism handling, maintenance
- QC failure investigations
  - Lessons learned


QC organism
• Organism viability
• Purity
• Incubation conditions (environment)
Test process (Human or test error)
• Incubation conditions (environment)
• Incubation time
• Training/gaps
- Result interpretation
- Sterile technique
Reagent issues (Media or Antibiotic)
• Reagent storage/integrity
• Expired reagents
• Media integrity (depth, cracked, contaminated)

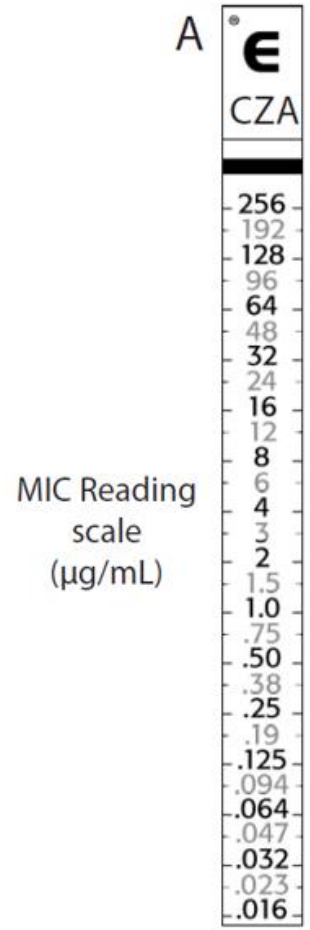
AST BENCH TRAINING

- Heavily automated, historically treated as an "easy" straightforward bench
  - Lack of training program, only taught "what" not "why"
  - Unclear policies
  - Turnover of senior/experienced techs, loss of knowledge
  - Observed increase in AST QC errors and failures

- Improved policies with added training/awareness
  - Prevent drift in procedures
- Training guides developed
- Improved QC documentation, problem logs

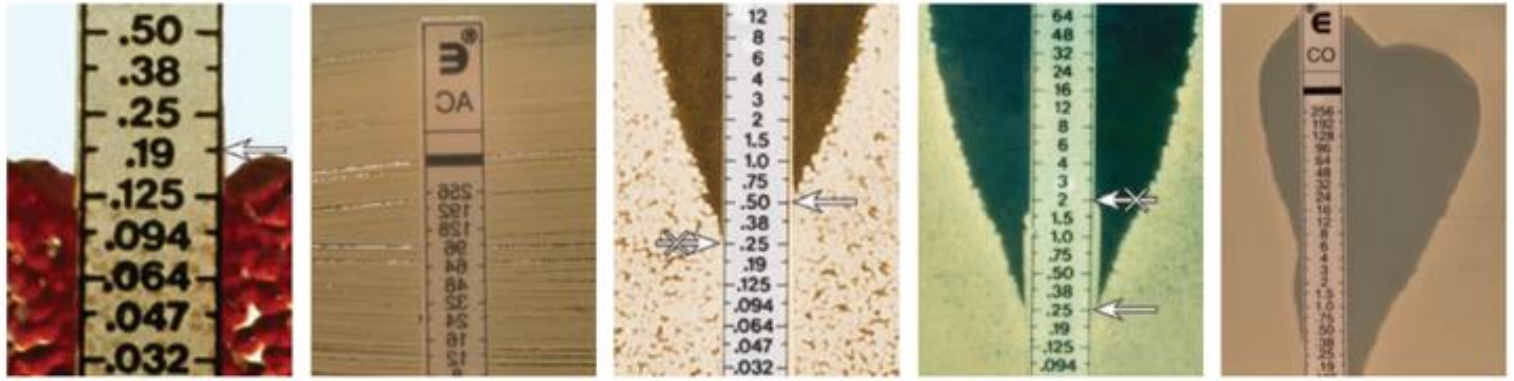
TRAINING GUIDES

- Manual reading of disks and strips
  - CLSI Disk Diffusion Reading Guide (eCLIPSE, clsi.org)
  - Etest Reading Guide (bioMeriuex)
    - Organism, drug effects
    - Resistance effects
  - Reporting 2-fold dilutions



MIC VALUES TO REPORT	
256	32
192	24
128	16
96	12
64	8
48	6
32	4
24	3
16	2
12	1.5
8	1.0
6	.75
4	.50
3	.38
2	.25
1.5	.19
1.0	.125
.75	.094
.50	.064
.38	.047
.25	.032
.19	.023
.125	.016
.094	.012
.064	.008
.047	.006
.032	.004
.023	.003
.016	.002

Technical and Handling:



Between markings – read upper value; **0.19** µg/mL

Etest strip placed upside down Invalid; repeat the test

Uneven – read upper value; if >1 dilution, repeat the test

Ignore line of growth alongside strip; **0.25** µg/mL

Distorted ellipse – wet surface, invalid; repeat the test

MEDICAL COLLEGE OF WISCONSIN

Search

CLSI M02 ED14 QG-2024

Document # Text

CLSI M02 ED14 QG-2024

Go To: Top | Bottom

Table of Contents

Section(s):

Figure(s):

QUICK GUIDE

M02-Ed14-QG

CLSI

Disk Diffusion Reading Guide

NOTE: Black or dashed lines throughout this guide indicate where the zone of inhibition should be measured.

General Rules for Measuring Zones of Inhibition (Figures 1 to 5)

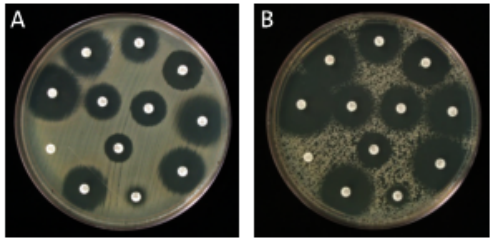


Figure 1. Assessing Growth

- Read plates only when the lawn of growth is confluent (Figure 1A).
- Repeat the test when individual colonies are apparent (Figure 1B).

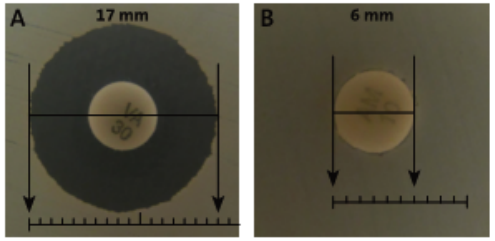


Figure 2. Measuring the Zones of Inhibition

- Measure zones of inhibition to the nearest whole millimeter (mm).
- Zones of complete inhibition include the diameter of the disk and show no obvious, visible growth as judged by the unaided eye (Figure 2A is measured as 17 mm); see Figures 6 to 10 for exceptions.
- Measure growth with no zone of inhibition as 6 mm (Figure 2B).
- Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibition.

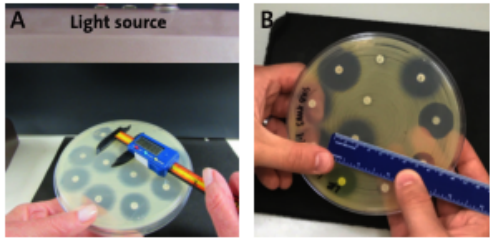
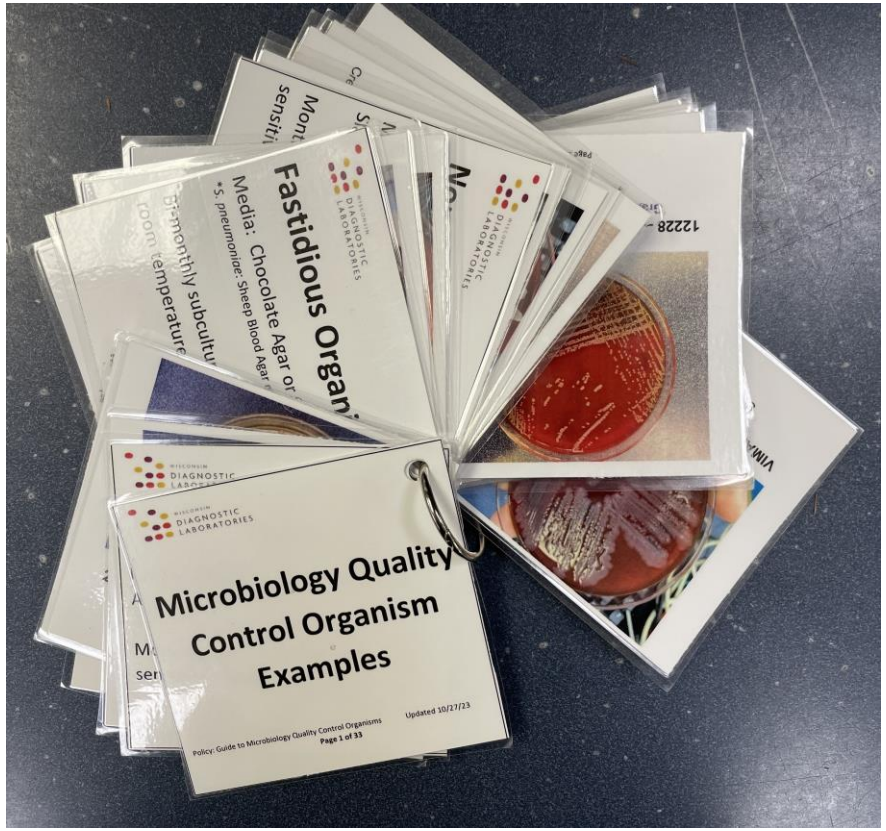


Figure 3. Measuring Zones of Inhibition Using Reflected Light and Translucent Media

- Invert the Petri plate and hold it a few inches above a black background that does not reflect light. Use a light source above the plate to read the zones.
- Measure complete zones of inhibition from the back of the inverted Petri plate (Figures 3A and 3B).

TRAINING GUIDES

- Flowcharts for QC organism subculture
  - Pre-printed labels for QC subcultures
- Organism morphology flashcards
- Sterile technique, handling organisms



QC DOCUMENTATION

- Improved QC failure documentation for better tracking of trends
  - Data input to spreadsheet for easier IQCP review
- Forms streamlined for consistency among techs
  - Selection of common errors
- Improved real time communication
  - AST QC issues discussed with team at daily huddles
  - Leadership review of manual AST

- Previous QC problem logs gave minimal information
  - "Out of range", "Reset up"
  - Delays in investigation and resolution

SELECT ONE - PANEL TYPE OUT OF CONTROL:

☐ NMIC-306 ☐ PMIC-110 ☐ SMIC-101 ☐ GN Manual Panel ☐ Kirby-Bauer Panel

QC ORGANISM: _____ **ANTIBIOTIC(S):** _____

**Refer to original/attached QC sheet for lot numbers and expiration dates*

SELECT THE ERROR OR FAILURE REASON BELOW:

☐ Mixed or contaminated growth ☐ Not set up or incubated correctly ☐ Other (specify below)

☐ Result(s) out of range Expected range: _____ Actual result: _____

DETAILED DESCRIPTION OF ERROR OR FAILURE (REQUIRED):

- Set yourself up for success
  - Can't go back in time, plates can be overgrown or discarded
  - Staff must thoroughly document incident at time it occurs

- Clear, easy to use forms includes information needed for QC investigation
- Allows techs to consistently document details of incident

CORRECTIVE ACTION TAKEN:

New organism subcultured from frozen? ☐ YES ☐ NO

Testing repeated -- Set Up Date: _____ Set Up Tech: _____

Read Out Date: _____ Read Out Tech: _____

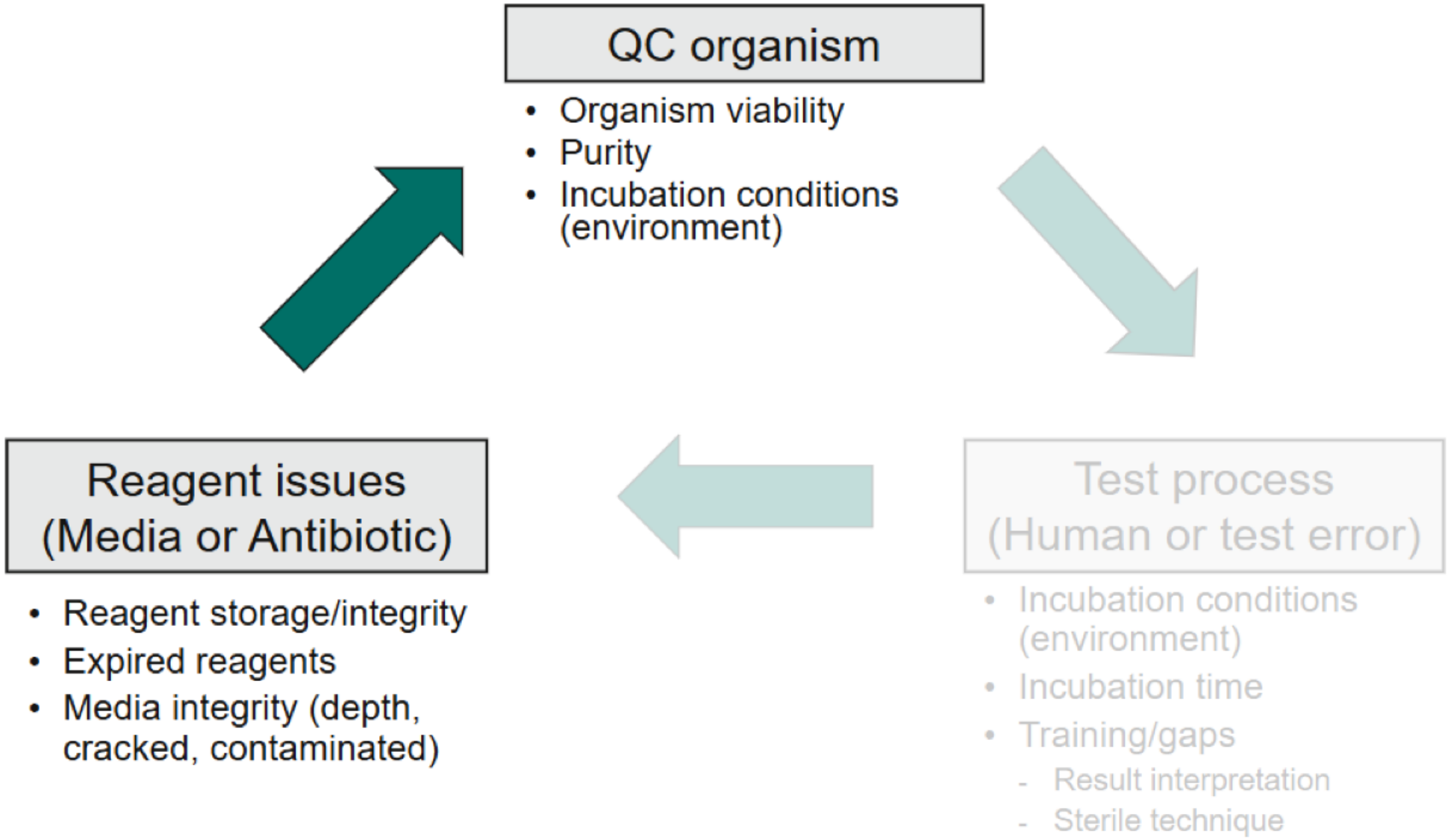
Result of repeat testing: _____

☐ Results in control, resume patient testing & reporting

☐ Repeat testing FAILED, begin 5 DAY QC and notify leadership immediately

NOTE: Attach 5 DAY QC form to this problem log

LEADERSHIP REVIEW & NOTES: ☐ Random Error ☐ Identifiable Error ☐ System Error


QC organism
• Organism viability
• Purity
• Incubation conditions (environment)
Test process (Human or test error)
• Incubation conditions (environment)
• Incubation time
• Training/gaps
- Result interpretation
- Sterile technique
Reagent issues (Media or Antibiotic)
• Reagent storage/integrity
• Expired reagents
• Media integrity (depth, cracked, contaminated)

QC FAILURE AND TROUBLESHOOTING

- QC failures: What to consider
  - Was the correct organism or reagent used?
  - Is the tested isolate pure?
  - Correct incubation time?
  - Correct incubation conditions?
  - Was the standard inoculum used?
  - Was the test interpreted appropriately?
  - Is there a problem with stock organisms?

CORRECT ORGANISM OR REAGENT

- Time of testing failure for anaerobic susceptibilities with penicillin
  - Expected range for *B. fragilis* ATCC 25825 is 8-32 µg/mL
  - Results were consistently >32 µg/mL
- Root cause investigation led to a review of the package inserts
  - Penicillin (32) - Indications for use do not include anaerobes
  - Penicillin (256) - Indications for use do include anaerobes
- QC passed once penicillin (256) use was implemented for anaerobic susceptibilities

ISOLATE LEVEL PURITY

- Weekly QC started showing failure for ceftazidime and pip/tazo with *P. aeruginosa* ATCC 27853
  - Purity plate and MH agar showed two different morphotypes
- Subcultured from frozen working stock showed two morphotypes again
  - Provides evidence that the failure may be due to contamination
- Possible delay in results, but affiliated institutes were utilized (CHW)
  - Pure isolate of the same ATCC strain was used to prevent further testing delays
- New Culti-Loop used and new working stock made.
- Likely source of error was contamination while subculturing the Bi-weekly isolates

CORRECT INCUBATION TIME

- Time of testing for nitrofurantoin on *Staphylococcus* species on urine sources kept failing due to an increased zone of inhibition
- Panel was read out at the beginning of the shift and re-incubated until the end of the shift.
  - Set up requiring more manual input usually performed towards the end of the shift
  - Zone was now within range
  - Troubleshooting steps were successfully documented, allowing for leadership intervention to ensure QC was read after correct incubation time requirements

MEDIA DISCREPANCIES

- Noted increased resistance of *Cutibacterium* ssp. to penicillin.
  - Isolates were sent to a reference laboratory for confirmation, all confirmed with susceptible results
  - Only factor not ruled out was the media
    - Commercially purchased individually wrapped, pre-reduced Brucella agar was used
  - A different manufacturer of Brucella agar was obtained and set up side by side with the previously used agar, susceptible results were observed with this different brand of media

STANDARD INOCULUM

- Infrequent and random weekly QC failures
  - No common trend was noted (i.e. not the same "bug/drug" combination failing)
  - Failures were only on manual panel QC
- Possible reason for failure could be variable inoculum density
  - Implemented use of the AP to ensure every sample was at a 0.5 McFarland

APPROPRIATE INTERPRETATION

- Failures noted when testing minocycline with *E. coli* ATCC 25922
  - Removal of minocycline from weekly QC to TOT
- Documentation showed the same failure. MIC values were consistently one dilution too high
  - Minocycline is bacteriostatic and was not being interpreted correctly
  - When the test was being read at 80% inhibition, QC was successful

QC STOCK: CREATION AND MAINTENANCE

- QC Stock Organisms:
  - Best practices
    - Ensure the stock was made appropriately
      - Made from the first subculture
      - Inoculated into sufficient volume
      - Thoroughly homogenized
    - Ensure the stock is used appropriately
      - Ultra-low temperatures are maintained
      - Subculturing is done appropriately per organism requirements
      - Systems in place that work best for the lab doing testing

CREATING A STOCK

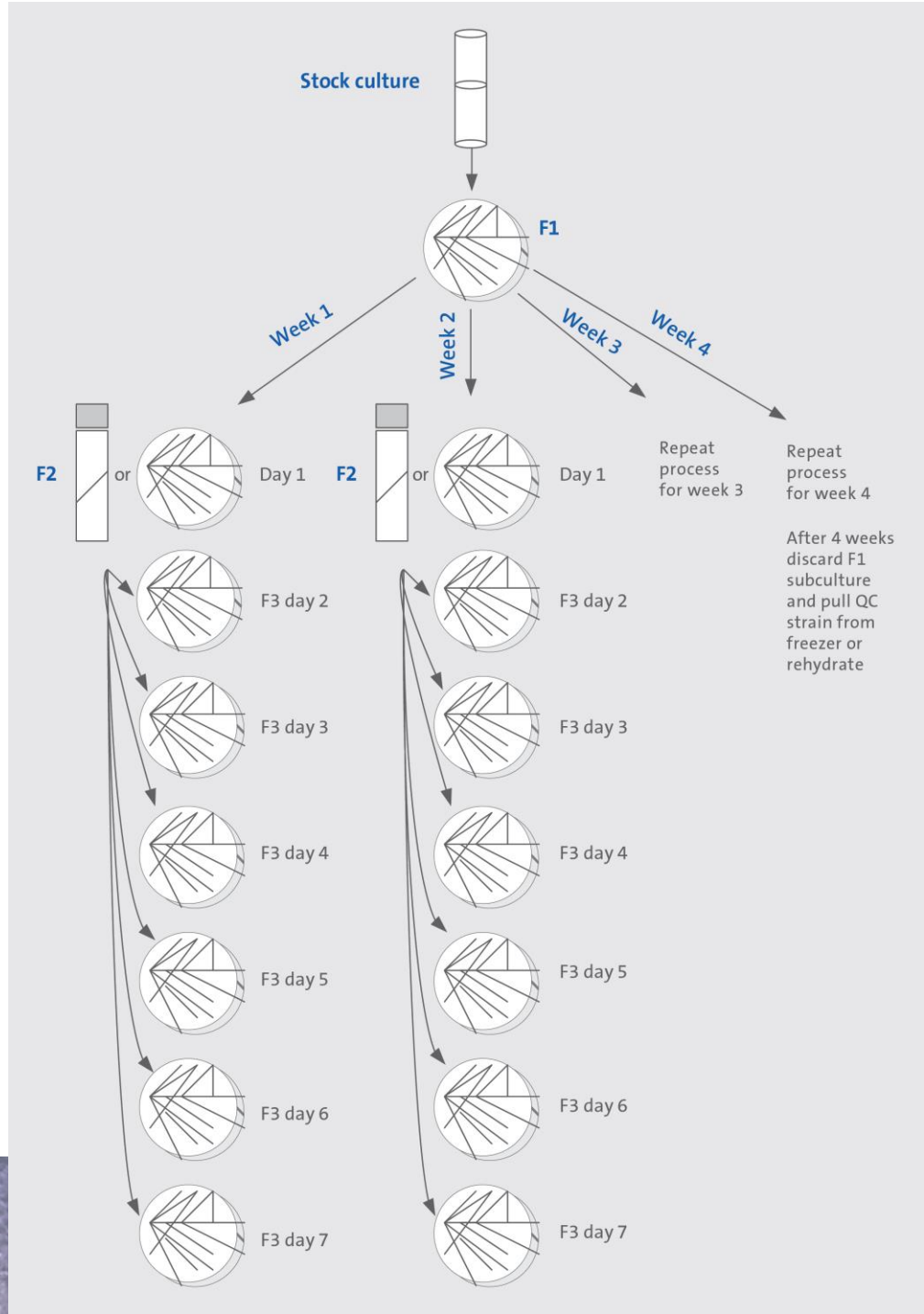
- Always create a stock from the first subculture of the organism or strain in question
  - Serial subculturing can affect AMR genes
- Use the appropriate volume if using a liquid based storage system
  - Low volumes of glycerol not effective at preventing crystallization
- Make sure the sample is homogenized prior to storage
  - Ensures successful subculturing

MAINTAINING A STOCK

- Ultra-low temperatures are maintained
  - Freeze-thaw cycles can have adverse activity on AMR genes
- Subculture appropriately
  - Bi-Weekly versus monthly subculturing
  - Fastidious organism subculturing
- Use what works best for your lab. What works for one may not work for the other.
  - Labeling
  - Aliquots

CLSI M02

- Workflow for subculturing and using reference strains
  - Figure C1



SUMMARY

- QC must be performed daily
  - QC frequency can be reduced if performance is acceptable and IQCP is in place
- AST training
  - Not the easy bench
  - Additional training and resources to support policies
- QC failure documentation
  - Improve QC failure documents to aid in investigation/tracking
- QC failure troubleshooting and strain maintenance
  - Investigate multiple possibilities to find the cause of QC error
  - Organization of QC stocks and subcultures can support fastidious organisms, reduce plasmid loss, and reduce QC errors

RESOURCES

- QC frequency, maintenance, troubleshooting
  - CLSI M02-ED14:2024
    - Performance Standards for Antimicrobial Disk Susceptibility Tests
- IQCP
  - cap.org (e-LAB solutions suite)
  - cdc.gov (https://www.cdc.gov/lab-quality/docs/developing-iqcp.pdf)
  - asm.org (https://asm.org/Protocols/Individualized-Quality-Control-Plan-IQCP)
- QC recommendations and ranges
  - CLSI M100, M45, Package inserts

MCW VALUES

We strive for excellence in education, research, patient care, and community engagement by:

car•ing
acting in **caring** ways

col•lab•o•ra•tive
engaging in **collaborative** efforts

cu•ri•os•i•ty
approaching our world with **curiosity**

in•clu•sive
advancing **inclusive** practices

in•teg•ri•ty
demonstrating **integrity** in all we do

re•spect
treating everyone with **respect**