



LABORATORY TRAINING SERIES

# Antimicrobial Susceptibility Testing (AST)

A Foundational Guide for Laboratorians

*From how antibiotics work, to how the laboratory determines which ones will work for a specific patient.*

**Lindsey E Nielsen, PhD, D(ABMM)**

July 2026



● ORIENTATION

# Learning Objectives

*By the end of this session, you should be able to:*

## 1 Explain the purpose of AST

Describe why clinical laboratories test bacterial and yeast isolates against antimicrobial agents, and what the result is used for.

## 2 Compare the reference methods

Distinguish broth microdilution, disk and gradient diffusion, and automated AST and understand how commercial platforms relate back to them.

## 3 Apply testing criteria

Recognize when AST is indicated — and when it is not — based on organism, specimen, and clinical context.

## 4 Interpret S / I / R results

Explain what a breakpoint is, how it is set, and why interpretation is organism- and drug-specific.



## ● WHY IT MATTERS

# One Test Result, One Treatment Decision

*AST results go directly into a prescribing decision — often for a patient who is already sick.*

**4.9M+**

antimicrobial-resistant infections occur in the U.S. each year

**1.27M+**

deaths per year are attributed to antimicrobial-resistant infections

**1 in 6**

Proportion of clinical isolates are antibiotic resistant

**The laboratory is the bridge between the organism and the prescription.** A correct, timely AST result lets a clinician narrow therapy to the most effective, least toxic, least ecologically disruptive agent or escalate quickly when resistance is present.

WHO CLASS data, 2026



## ● THE BASICS

# What Is Antimicrobial Susceptibility Testing?

AST is a laboratory test that determines whether a specific antimicrobial agent is likely to inhibit or kill a specific organism **isolated from a patient.**

*It is performed in vitro (“in glass” / in the lab) and used to predict the likely in vivo (in the patient) response.*

## Organisms Routinely Tested



### Bacteria

The great majority of AST volume — aerobic and, less commonly, anaerobic isolates



### Yeast

Candida and related species, using antifungal susceptibility methods



### Filamentous fungi (rare)

Reference antifungal methods exist, but testing is largely centralized to reference labs

## The Core Question AST Answers

*“At concentrations this drug can safely achieve in the patient, will it stop this specific organism from growing?”*



## ● PHARMACOLOGY REFRESHER

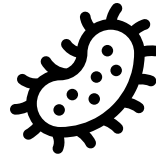
# How Antibiotics Work: Targets

*Every antibiotic class exploits a structural or metabolic difference between bacteria and human cells.*



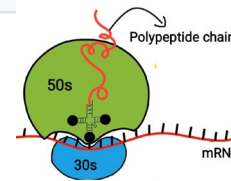
### Cell Wall Synthesis

Penicillins, cephalosporins, carbapenems, monobactams, glycopeptides



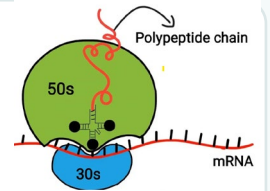
### Protein Synthesis (30S)

Aminoglycosides, tetracyclines



### Protein Synthesis (50S)

Macrolides, lincosamides, oxazolidinones, streptogramins



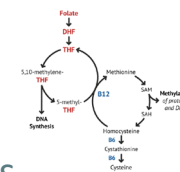
### Nucleic Acid Synthesis

Fluoroquinolones (DNA), rifamycins (RNA)



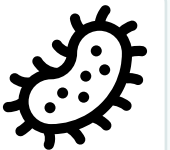
### Folate / Metabolic Pathway

Trimethoprim, sulfonamides



### Cell Membrane Integrity

Polymyxins, lipopeptides (e.g., daptomycin)





# How Bacteria Fight Back: Resistance Mechanisms

## 1 Enzymatic Inactivation

The organism produces an enzyme that destroys or modifies the drug before it reaches its target.

*e.g., beta-lactamases inactivating penicillins/cephalosporins*

## 2 Target Alteration

The drug's binding site is mutated or modified so the drug can no longer bind effectively.

*e.g., altered PBPs in methicillin-resistant staphylococci*

## 3 Reduced Uptake / Efflux

The organism limits how much drug gets in (porin loss) or actively pumps it back out.

*e.g., efflux pumps in many gram-negative organisms*

## 4 Pathway Bypass

The organism develops an alternate metabolic route that avoids the blocked step entirely.

*e.g., acquired alternate folate pathway enzymes*



● A BRIEF HISTORY

# From Fleming's Petri Dish to a Standardized Test



<https://www.businessinsider.com/>

1928–29

Fleming observes a *Penicillium* mold inhibiting bacterial growth on a contaminated plate — the discovery that launches the antibiotic era.

1940s–50s

Antibiotics enter widespread clinical use. Individual laboratories develop their own, non-standardized susceptibility methods.

1950s–60s

Resistant organisms emerge almost as quickly as new drugs. Results vary lab-to-lab, making outcomes hard to compare or trust.

1960s–present

Standards organizations (CLSI in the U.S.; EUCAST internationally) formalize reproducible methods, quality control, and breakpoints.

*The lesson that shaped modern AST: an antibiotic must be tested “against the very organism it is intended to treat,” and the result is only useful if it is reproducible from one laboratory to the next.*

Fleming A. On the antibacterial action of cultures of a *penicillium*. *Br J Exp Pathol*. 1929;10(3):226–236.



## ● A BRIEF HISTORY

# Standardizing the Test: CLSI and EUCAST

## CLSI

*Clinical and Laboratory Standards Institute*

- Publishes the reference methods used in most U.S. laboratories (disk diffusion and broth dilution standards)
- Publishes and updates interpretive breakpoints for common bacteria annually in the M100 supplement
- Consensus process bringing together laboratorians, clinicians, industry, and regulators

## EUCAST

*European Committee on Antimicrobial Susceptibility Testing*

- Sets the parallel standard used across much of Europe and increasingly worldwide
- Publishes its own breakpoint tables, which can differ numerically from CLSI's
- Joint CLSI–EUCAST working groups coordinate on methodology where possible

*Why it matters on the bench: your instrument or panel is validated to one of these breakpoint sets. Know which one your laboratory reports — it is usually printed on your result report or SOP.*

CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*, 36th ed., M100. Clinical and Laboratory Standards Institute; 2026. EUCAST Breakpoint Tables ([eucast.org](https://www.eucast.org)).



## ● METHODS

# Two Reference Methods Underpin Everything

*Every method in the lab — manual or automated — is validated against one of these two.*



### Broth Microdilution

*Determines the Minimum Inhibitory Concentration (MIC)*

- Organism is exposed to serial two-fold dilutions of antibiotic in liquid growth medium
- Lowest concentration showing no visible growth = the MIC
- Reported as a concentration, in µg/mL
- The international reference standard; used by regulators as the benchmark method



### Disk Diffusion

*Determines a Zone of Inhibition*

- A paper disk saturated with a fixed antibiotic concentration is placed on an inoculated agar plate
- Antibiotic diffuses outward in a concentration gradient as the organism grows overnight
- The diameter of the clear zone around the disk is measured, in millimeters
- Zone size correlates back to an MIC range for interpretation

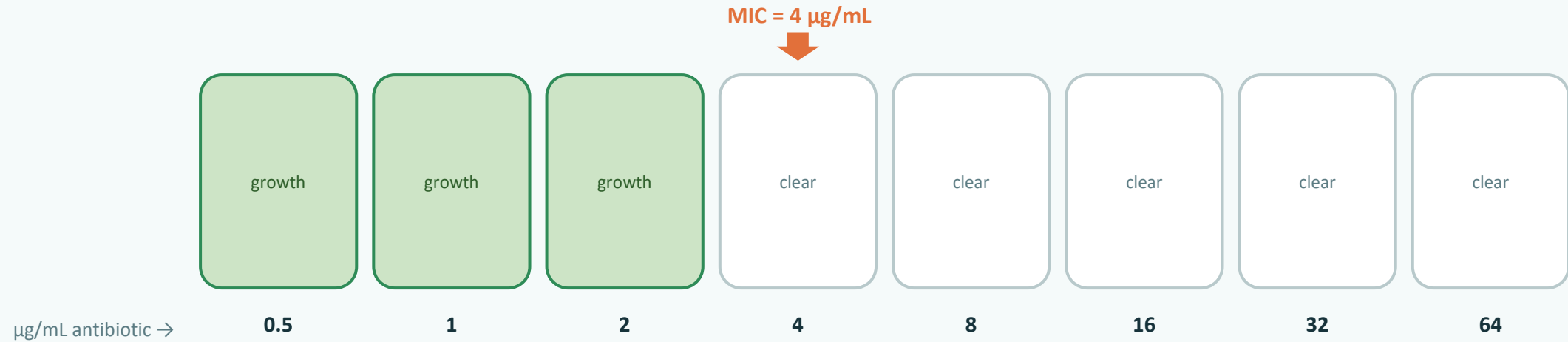
*CLSI M07 (dilution methods) and CLSI M02 (disk diffusion), Clinical and Laboratory Standards Institute.*



## ● METHODS — BROTH MICRოდILUTION

# The Minimum Inhibitory Concentration (MIC)

*The lowest antibiotic concentration that prevents visible growth after overnight incubation.*



- Growth control well (no antibiotic) must show growth, confirming the organism was viable and correctly inoculated
- The first clear well in the dilution series is the MIC — here, growth stops at 4 µg/mL, so the MIC is 4 µg/mL
- Standard test variability is  $\pm$  one two-fold dilution, which is expected and does not indicate an error

*CLSI M07: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically.*

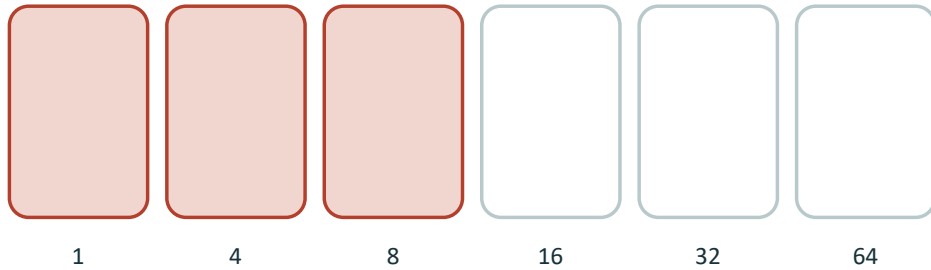


## ● METHODS — BROTH MICRODILUTION

# Reading the MIC: Two Organisms, Same Drug

### Isolate #1

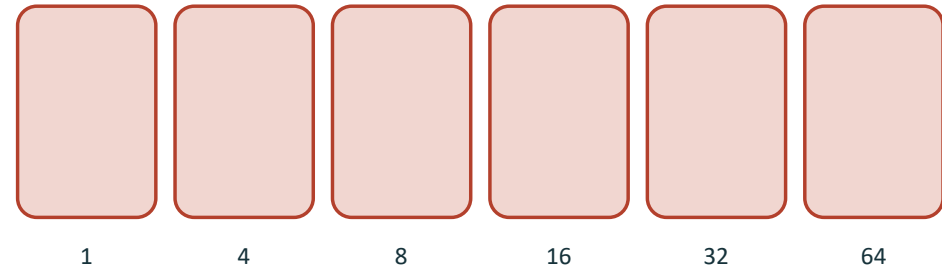
MIC = 16 µg/mL



*Growth stops early — a low concentration is enough to inhibit this organism.*

### Isolate #2

MIC > 64 µg/mL

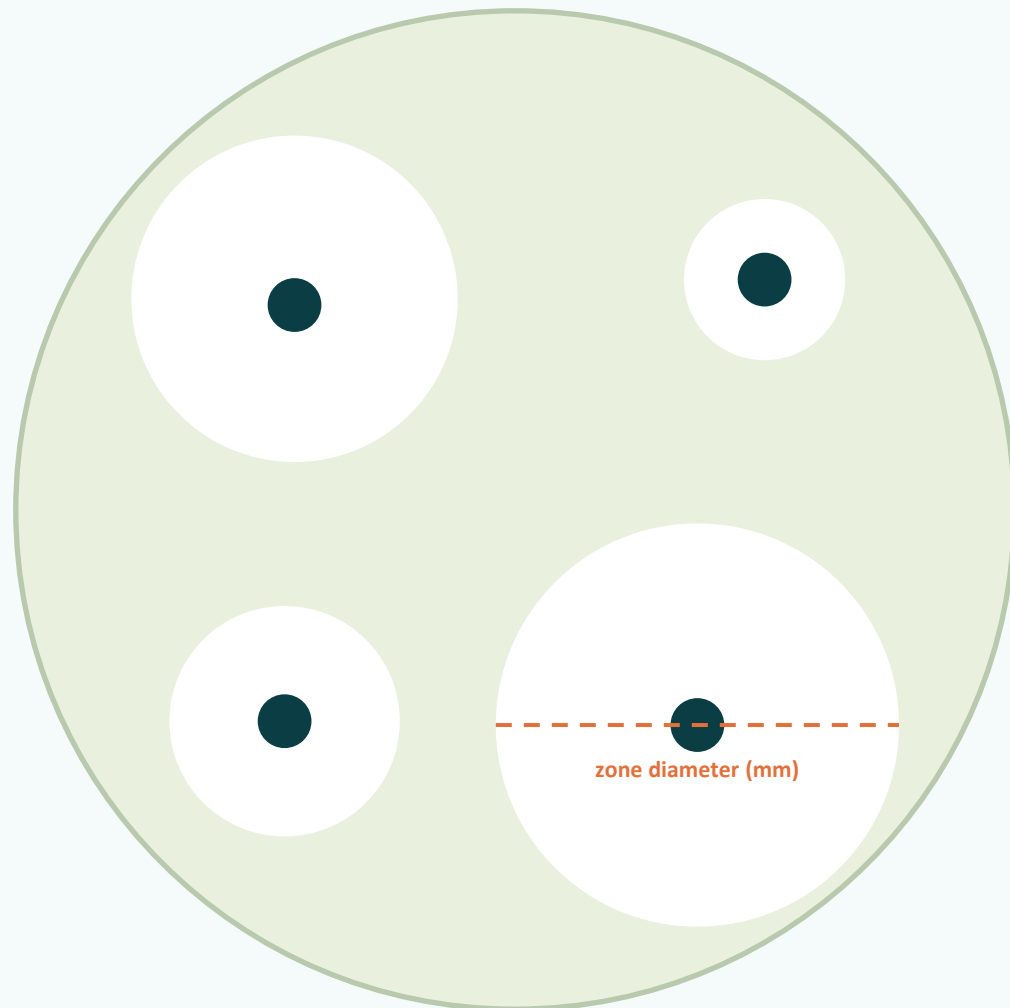


*Growth continues through the entire tested range — achievable drug levels will not work.*



## ● METHODS — DISK DIFFUSION

# The Disk Diffusion Test



- Agar plate is inoculated with a standardized suspension of the organism, forming a uniform lawn.
- Paper disks, each pre-loaded with a fixed concentration of one antibiotic, are placed on the surface.
- The antibiotic diffuses outward, creating a concentration gradient that decreases with distance.
- After overnight incubation, the zone of inhibition (clear area) is measured in millimeters and compared to a breakpoint table.
- Some agents cannot be reliably tested by disk diffusion — including several with poor or unpredictable agar diffusion — and require MIC-based methods instead.



● METHODS

# MIC vs. Disk Diffusion, Side by Side

|                                                     | Broth Microdilution (MIC)               | Disk Diffusion                                |
|-----------------------------------------------------|-----------------------------------------|-----------------------------------------------|
| Result reported as                                  | A concentration (µg/mL)                 | A distance (mm)                               |
| Format                                              | Liquid medium in a panel/tray           | Solid agar plate                              |
| Can calculate a specific dose relationship (PK/PD)? | Yes                                     | No — correlated back to MIC ranges            |
| Throughput on manual bench                          | Lower per-isolate labor with automation | Simple, inexpensive, flexible for many agents |
| Some agents excluded?                               | Rare                                    | Yes — poor diffusers must use MIC methods     |

CLSI M02 and M07, Clinical and Laboratory Standards Institute.



## ● METHODS

# Commercial AST Platforms: Same Principles, Different Format

*Most laboratories do not run manual reference-method testing for every isolate — they use a validated commercial system.*

1

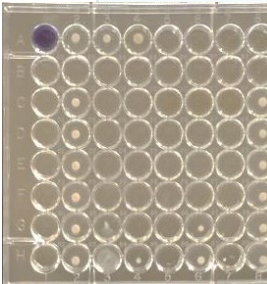
### Automated broth microdilution systems

Instrument-read microdilution panels that report an MIC (and derived S/I/R) for a full antibiotic panel, often within hours.

2

### Manual broth microdilution panels

Pre-made dilution trays read visually or with a simple reader; same underlying principle as automated systems.



3

### Gradient diffusion strips

A plastic strip with a continuous antibiotic gradient replaces the disk, giving a direct MIC readout from an agar plate.

4

### Disk diffusion

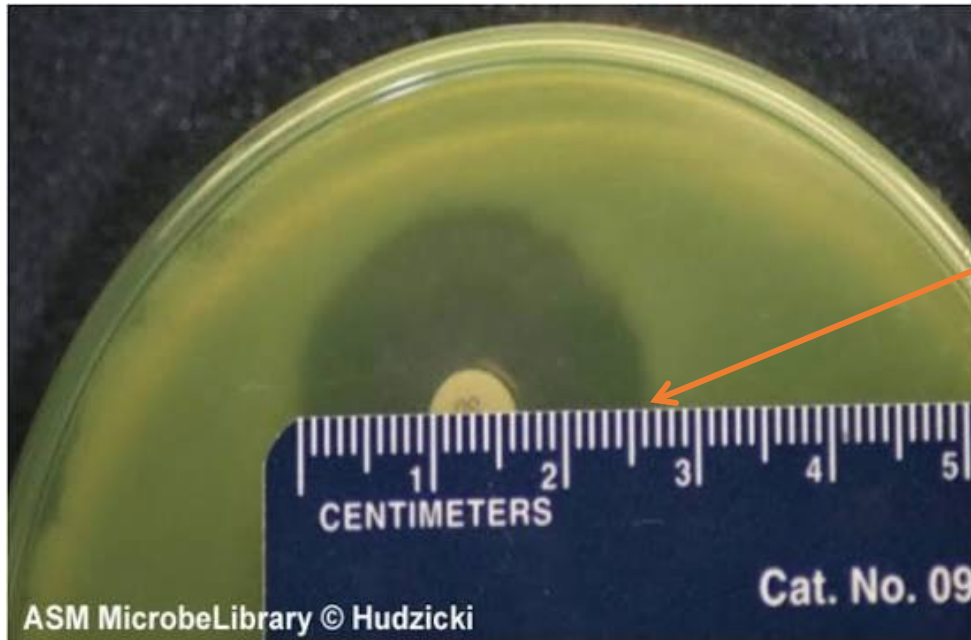
The original reference method, still routinely used, especially for organisms or agents not well suited to panels.

*In the U.S., a commercial system must be FDA-cleared, demonstrating acceptable agreement with the CLSI reference method, before a laboratory can use it for patient results.  
In other countries, this is done through local regulatory agencies and demonstrated through CE-markings*



● METHODS

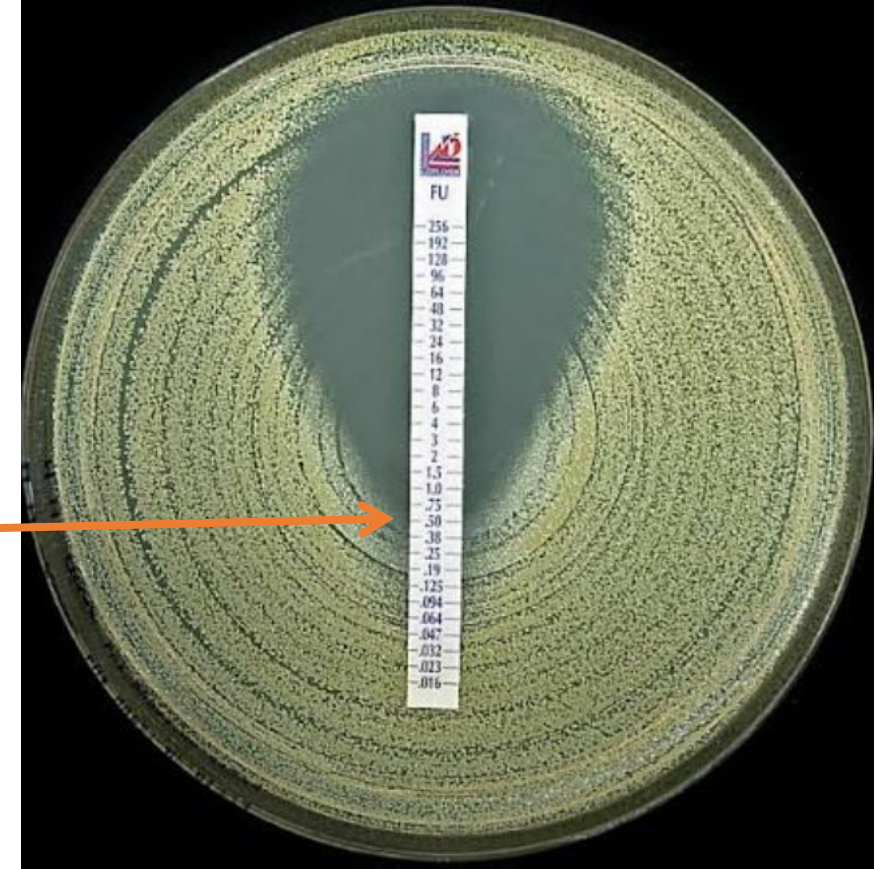
# Gradient Diffusion Disc vs. Strips



Zone Diameter  
26mm

Vs

MIC of 0.5



Liofilchem.com



## ● GETTING IT RIGHT

# Principles Every AST Method Depends On

*The method only predicts reality if these variables are controlled — this is where technique matters most.*

### Pure Culture

Testing must start from a single, pure isolate — a mixed culture invalidates the result.

### Growth Phase

Organisms should be in log-phase (actively dividing) growth, not an old or stressed culture.

### Inoculum Density

A standardized suspension (typically referenced to a 0.5 McFarland turbidity standard) ensures a consistent starting concentration.

### Incubation Time

Most panels are read at 16–20 hours; some organisms require 24 hours — reading too early or late skews results.

### Temperature

Standard incubation is  $35\pm 2^{\circ}\text{C}$  for most routine testing.

### Atmosphere

Follow the manufacturer's package insert for the specific drug-organism combination as some organisms require a specific atmosphere for reliable growth or test conditions.

*CLSI M02 and M07, Clinical and Laboratory Standards Institute.*



● CLINICAL JUDGMENT

# When Should AST Be Performed?



**The organism is recovered in culture**

*in adequate quantity from an appropriate specimen*



**It is a plausible pathogen**

*for the anatomical site the specimen came from, not an expected commensal*



**Susceptibility is not predictable**

*the organism does not have a well-established, reliably predictable susceptibility pattern*



**Your AST inoculum is a pure culture**

*so the result can be attributed to a specific organism with confidence*



# When AST Is Not Necessary — or Not Appropriate

## Predictably Susceptible Organisms

- Some organisms remain reliably susceptible to standard first-line agents — testing adds cost and delay without changing management.
- Example: Group A Streptococcus remains universally susceptible to penicillin; routine AST is not necessary for this pairing.
- Reporting an unnecessary result can also tempt broader-spectrum prescribing than needed.

## Likely Contaminated Specimens

- Multiple organisms at low-to-moderate colony counts, especially from a non-sterile site, often represent contamination or colonization rather than infection.
- Example: a urine culture with several organism types at modest counts, including skin/genital flora, more often reflects collection technique than a true UTI.
- Testing (and reporting) here risks treating a contaminant — a repeat, properly collected specimen is usually the better call.

Caveat: Sometime an organism requires AST results the first time it is isolated but not if it isolated from multiple specimens at the same time or within a short time frame of each other. In this case, lab reports often refer back to the isolate first tested – consult your Standard Operating Procedure!



## ● CLINICAL JUDGMENT

# Selecting the Isolate: A Urine Culture Example

### Perform AST

*> 100,000 CFU/mL Escherichia coli, no other organisms recovered*

- Significant quantity of a well-established urinary pathogen
- No competing organisms suggesting contamination
- Result will directly guide antibiotic selection for a likely true UTI

### Do Not Perform AST

*Mixed growth: Corynebacterium spp., E. coli, yeast, and Lactobacillus spp., all at modest counts*

- Multiple organism types, several of which are typical genital/periurethral flora
- Pattern is classic for a poorly collected (contaminated) specimen
- Recommend a freshly and properly collected specimen if a UTI is still suspected



## ● INTERPRETING RESULTS

# How AST Is Reported: S, I, R



### Susceptible

*MIC falls below the susceptible breakpoint.*

The antimicrobial agent is expected to inhibit the pathogen at standard dosing — predicted clinical success.



### Intermediate / Susceptible-Dose-Dependent

*MIC falls in the intermediate range, near the breakpoint.*

May still work if the dose is increased, or if the infection is at a site where the drug concentrates — a conditional “maybe.”



### Resistant

*MIC exceeds the resistant breakpoint.*

The antimicrobial agent is not expected to inhibit the pathogen at achievable levels — predicted clinical failure.

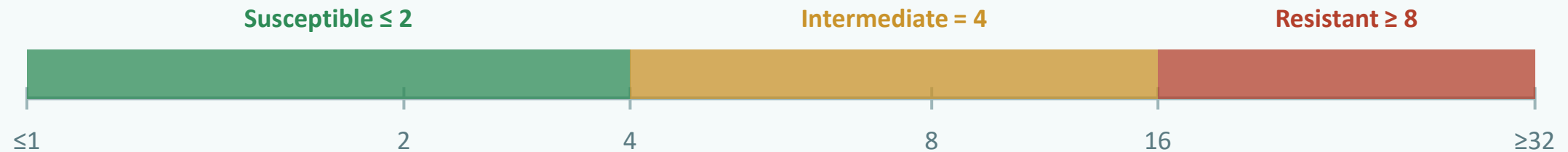


## ● INTERPRETING RESULTS

# What Is a Breakpoint?

*A breakpoint is a specific MIC (or zone diameter) value chosen to divide results into susceptible, intermediate / SDD, and resistant categories for a given organism–drug combination.*

*A simplified example — not an actual published breakpoint:*



*Breakpoints are not fixed physical constants — they are set (and periodically revised) by expert committees, and they differ by organism, by drug, and sometimes by infection site.*

CLSI M100, 36th ed., Clinical and Laboratory Standards Institute; 2026.



## ● INTERPRETING RESULTS

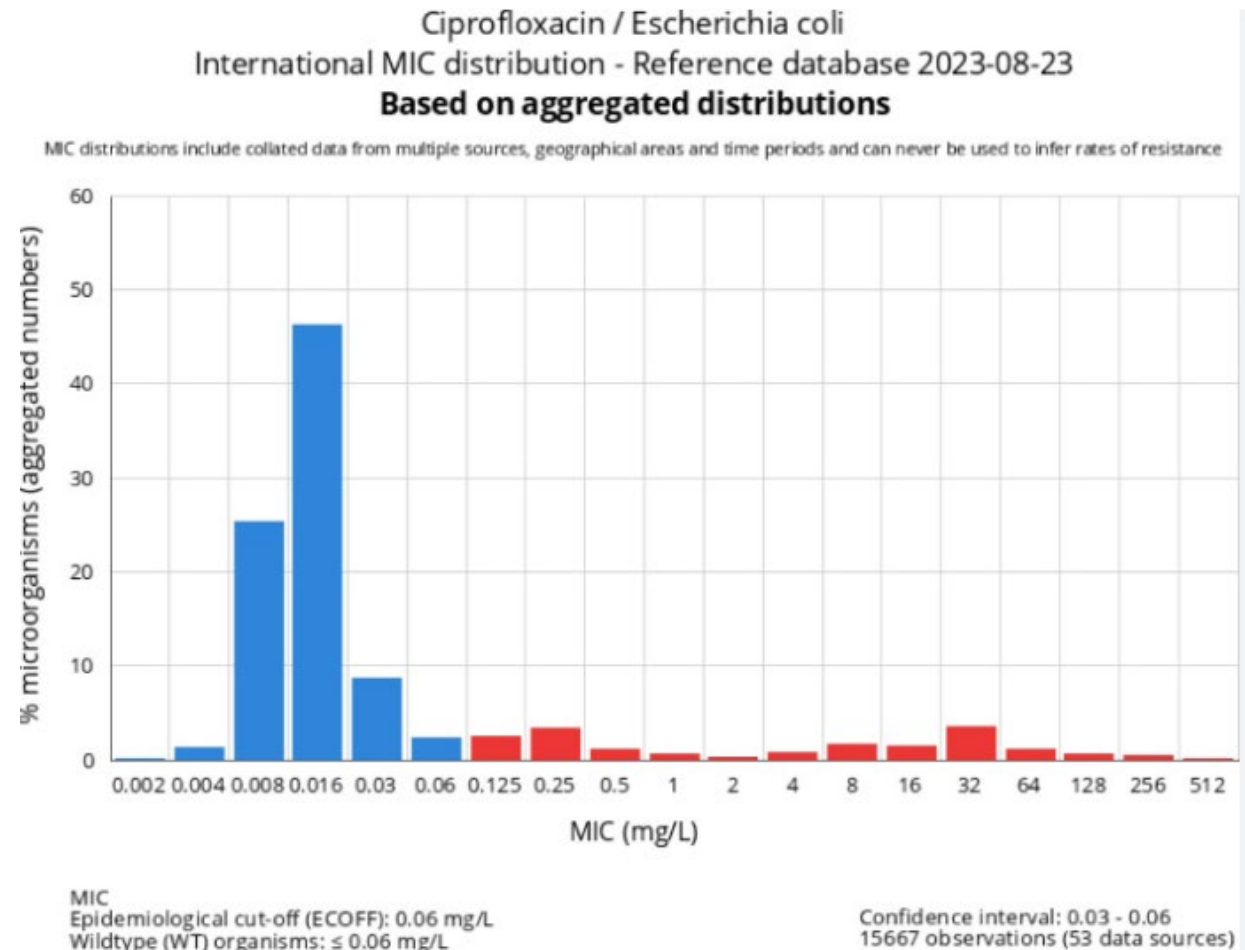
# How Breakpoints Are Set: Three Pillars

*Committees weigh three independent lines of evidence before setting or revising a breakpoint.*

1

## Microbiological Data

MIC distributions across large populations of the organism define the “wild-type” (no acquired resistance) range versus organisms with acquired resistance mechanisms.



<https://pmc.ncbi.nlm.nih.gov/articles/PMC10732016/>



## ● INTERPRETING RESULTS

# How Breakpoints Are Set: Three Pillars

*Committees weigh three independent lines of evidence before setting or revising a breakpoint.*

1

### Microbiological Data

MIC distributions across large populations of the organism define the “wild-type” (no acquired resistance) range versus organisms with acquired resistance mechanisms.

2

### Pharmacokinetic / Pharmacodynamic (PK/PD) Data

Modeling of how much drug reaches the infection site, and for how long, given doses that are safe and achievable in patients.



## ● INTERPRETING RESULTS

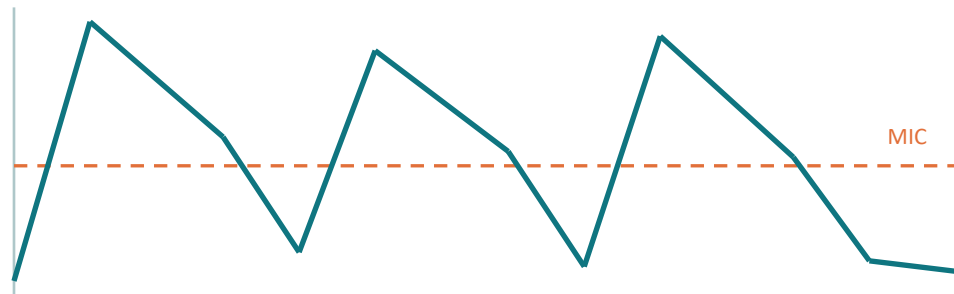
# PK/PD Basics: Two Killing Patterns

*Different antibiotic classes need different exposure patterns to kill bacteria effectively.*

## Time-Dependent Killing

*e.g., beta-lactams*

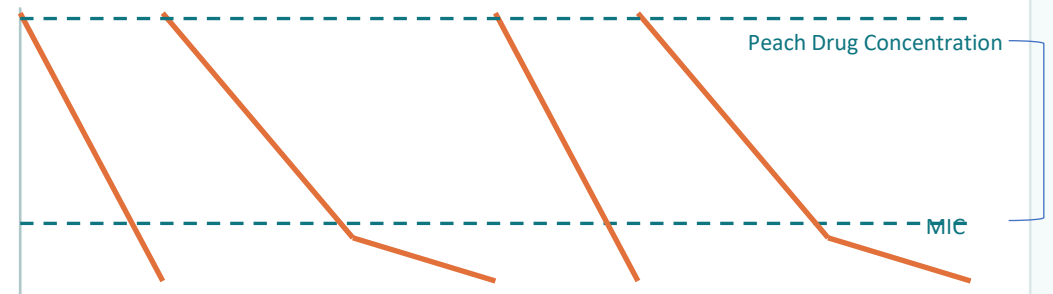
Goal: maximize the % of the dosing interval that levels stay above the MIC → favors frequent or extended dosing



## Concentration-Dependent Killing

*e.g., aminoglycosides, fluoroquinolones*

Goal: maximize the peak concentration relative to the MIC → favors larger, less frequent doses



*Adapted from Craig WA. Clin Infect Dis. 1998;26(1):1–10.*



## ● INTERPRETING RESULTS

# How Breakpoints Are Set: Three Pillars

*Committees weigh three independent lines of evidence before setting or revising a breakpoint.*

1

### Microbiological Data

MIC distributions across large populations of the organism define the “wild-type” (no acquired resistance) range versus organisms with acquired resistance mechanisms.

2

### Pharmacokinetic / Pharmacodynamic (PK/PD) Data

Modeling of how much drug reaches the infection site, and for how long, given doses that are safe and achievable in patients.

3

### Clinical Outcome Data

Real-world data linking MIC values to actual treatment success or failure in treated patients.



## ● INTERPRETING RESULTS

# How Breakpoints Are Set: Three Pillars

*Committees weigh three independent lines of evidence before setting or revising a breakpoint.*

➤ [Lancet Infect Dis.](#) 2026 Jul;26(7):659-670. doi: 10.1016/S1473-3099(26)00020-4. Epub 2026 Mar 25.

## Survival trends in patients with difficult-to-treat, antibiotic-resistant, Gram-negative infections in the era of next-generation antibiotics in the USA: a retrospective cohort study

Morgan K Walker<sup>1</sup>, Christina Yek<sup>2</sup>, Sadia Sarzynski<sup>3</sup>, Sarah Warner<sup>1</sup>, Anthony D Harris<sup>4</sup>, Jonathan D Baghdadi<sup>4</sup>, Katherine E Goodman<sup>4</sup>, John H Powers 3rd<sup>5</sup>, Michael Klompas<sup>6</sup>, Chanu Rhee<sup>6</sup>, Bruce Swihart<sup>3</sup>, Sameer S Kadri<sup>7</sup>;

NIH Antimicrobial Resistance Outcomes Research Initiative (NIH-ARORI)

Affiliations + expand

PMID: 41903557 PMCID: [PMC13268009](#) DOI: [10.1016/S1473-3099\(26\)00020-4](#) [↗](#)

3

### Clinical Outcome Data

Real-world data linking MIC values to actual treatment success or failure in treated patients.



## ● INTERPRETING RESULTS

# Breakpoints Are Organism- and Drug-Specific

*The same MIC can mean something different depending on which organism produced it.*

E

*Enterobacterales*

Sa

*Staphylococcus aureus*

CoNS

*Coagulase-negative Staphylococcus or S.O.S.A*

Pa

*Pseudomonas aeruginosa*

Es

*Enterococcus spp.*

Each organism group has its own interpretive tables because intrinsic biology, typical resistance mechanisms, and clinical outcome data all differ by species.

***Practical rule: never apply a breakpoint from one organism group to another, even if the drug name is identical — always confirm you are reading the correct table.***

CLSI M100, 36th ed., Clinical and Laboratory Standards Institute; 2026.



● CASE STUDY 1

# Applying S / I / R in Practice

## Clinical Vignette

A 35-year-old woman is admitted with urosepsis. **Blood cultures grow *Escherichia coli*.**

*The laboratory reports the following **MICs** from the isolate:*

|               |           |
|---------------|-----------|
| Ampicillin    | 8 µg/mL   |
| Ceftriaxone   | 0.5 µg/mL |
| Ciprofloxacin | 1 µg/mL   |

*Question: based on these MICs, which agent(s) are predicted to be active?*

## CASE STUDY 1

# Applying S / I / R in Practice

### Clinical Vignette

A 35-year-old woman is admitted with urosepsis. **Blood cultures grow *Escherichia coli*.**

*The laboratory reports the following MICs from the isolate:*

Ampicillin **8 µg/mL**

Ceftriaxone **0.5 µg/mL**

Ciprofloxacin **1 µg/mL**

*Question: based on these MICs, which agent(s) are predicted to be active?*

© Clinical and Laboratory Standards Institute. All rights reserved.

Table 2A-1. Enterobacterales (excluding *Salmonella* and *Shigella* spp.) (Continued)

- (4) Positive blood culture broth can be used as the inoculum for direct disk diffusion testing of select antimicrobial agents and methods described in Table 3F-1 and applying breakpoints in Table 3F-2. For antimicrobial agents not listed in Table 3F-2, not yet evaluated this direct disk diffusion method.

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

| Antimicrobial Agent | Disk Content | Interpretive Categories and Zone Diameter Breakpoints, Nearest Whole mm |     |                    |      | Interpretive Categories and MIC Breakpoints, µg/mL |     |                 |      |                                                                                                 |
|---------------------|--------------|-------------------------------------------------------------------------|-----|--------------------|------|----------------------------------------------------|-----|-----------------|------|-------------------------------------------------------------------------------------------------|
|                     |              | S                                                                       | SDD | I                  | R    | S                                                  | SDD | I               | R    |                                                                                                 |
|                     |              | PENICILLINS                                                             |     |                    |      |                                                    |     |                 |      |                                                                                                 |
| Ampicillin          | 10 µg        | ≥ 17                                                                    | —   | 14-16 <sup>^</sup> | ≤ 13 | ≤ 8                                                | —   | 16 <sup>^</sup> | ≥ 32 | (5) Results used to pro<br>(6) Breakp<br>is used are<br>uncomplic<br><i>coli</i> and <i>Pro</i> |

## ● CASE STUDY 1

# Applying S / I / R in Practice

### Clinical Vignette

A 35-year-old woman is admitted with urosepsis. **Blood cultures grow *Escherichia coli*.**

*The laboratory reports the following **MICs** from the isolate:*

Ampicillin **8 µg/mL**

Ceftriaxone **0.5 µg/mL**

Ciprofloxacin **1 µg/mL**

*Question: based on these MICs, which agent(s) are predicted to be active?*

### After Applying Breakpoints

Ampicillin 8 µg/mL **S**

Ceftriaxone 0.5 µg/mL **S**

Ciprofloxacin 1 µg/mL **S**

**All three agents test susceptible — so which one should be used?**

- The laboratory's job ends at reporting an accurate, correctly interpreted result
- The prescriber's choice among susceptible agents depends on factors AST doesn't capture: allergy history, other medications, source control, and a preference for the narrowest effective spectrum
- This is why AST supports — but does not replace — clinical decision-making



## ● SPECIAL RESISTANCE PHENOTYPES

# Select Resistance Phenotypes

- A single AST result rarely is useful without knowing the larger isolate profile
- The isolate AST profile can provide insight to resistance mechanisms and potential of transferring the mechanism to another organism
- In addition to Antibiotic testing, we can also perform screening tests using antibiotics to look for certain resistance mechanisms
- Recognition of common vs. uncommon AST profiles can help
  - Determine result accuracy
  - Inform level of Infection Control Interventions
  - Understanding shifts in local epidemiology
  - Identify outbreaks

*McConeghy KW, Bleasdale SC, Rodvold KA. Clin Infect Dis. 2013;57(12):1760–1765.*



## ● SPECIAL RESISTANCE PHENOTYPES

# *Staphylococcus aureus*: MRSA vs. MSSA

*Same species, Staphylococcus aureus — but the correct drug class depends entirely on methicillin susceptibility.*



### **MSSA — Methicillin-Susceptible**

- Beta-lactams (e.g., anti-staphylococcal penicillins, first-generation cephalosporins) are typically the most effective option
- For bloodstream infections, outcome data and potential side effects favor a beta-lactam over a higher tiered antibiotic, (e.g. vancomycin) when the isolate is susceptible



### **MRSA — Methicillin-Resistant**

- Beta-lactams are rendered ineffective (with rare drug-specific exceptions) and must be avoided
- Alternative agents (e.g., vancomycin, ceftaroline, or other non-beta-lactam options) are required, generally with somewhat less favorable outcomes than a beta-lactam would offer for a susceptible strain

***Bottom line: getting this call right, quickly, is one of the highest-stakes results a microbiology lab produces.***

*McConeghy KW, Bleasdale SC, Rodvold KA. Clin Infect Dis. 2013;57(12):1760–1765.*



- SPECIAL RESISTANCE PHENOTYPES

# Detecting Methicillin Resistance

1

## Cefoxitin Surrogate Testing

Cefoxitin is used as a surrogate marker because it more reliably induces detectable resistance than oxacillin itself — the result is then reported as oxacillin, not cefoxitin, since cefoxitin itself is not used therapeutically for staphylococci.

2

## Oxacillin MIC (with salt supplementation)

Direct testing against oxacillin using a higher salt concentration to improve detection of heteroresistant populations.

3

## Molecular or Antigen Detection

Direct detection of the *mecA* (or *mecC*) resistance gene, or the PBP2a protein it encodes, independent of phenotypic growth.

CLSI M100, 36th ed.; Swenson JM, et al. J Clin Microbiol. 2005;43(8):3818–3823.



## ● SPECIAL RESISTANCE PHENOTYPES

# Enterococcus: Intrinsic Resistance and Vancomycin Resistant Enterococcus (VRE)

### Intrinsically Resistant To:

- Cephalosporins
- Clindamycin
- Aminoglycosides (at standard/low-level testing)
- Trimethoprim-sulfamethoxazole (in vivo activity unreliable despite in vitro results)

*These results should never be reported as susceptible for Enterococcus, regardless of what an automated panel's raw MIC might suggest — intrinsic resistance overrides.*

### Species Differences Matter

- *E. faecalis* — usually susceptible to ampicillin/penicillin; can acquire vancomycin resistance
- *E. faecium* — usually resistant to ampicillin/penicillin; vancomycin resistance (VRE) is common in this species
- Species-level identification is therefore essential before interpreting the susceptibility profile



## ● SPECIAL RESISTANCE PHENOTYPES

# ESBLs and Carbapenemases in Enterobacterales

## Extended-Spectrum Beta-Lactamases (ESBLs)

- Enzymes that inactivate most penicillins, cephalosporins, and monobactams
- Typically remain susceptible to carbapenems, which is why carbapenems are frequently the treatment of choice for serious ESBL infections
- Detected phenotypically through characteristic resistance patterns on routine panels, often confirmed with dedicated testing

## Carbapenems Producing Enterobacterales (CPE) and Carbapenem Resistance Organisms (CRO)

- CPEs have enzymes that inactivate carbapenems such as Meropenem and Imipenem
  - Among the most concerning resistance mechanisms in gram-negative organisms
- Phenotypic confirmatory methods (such as the modified/EDTA-modified carbapenem inactivation methods) distinguish true carbapenemase production (CPE) from other causes of reduced susceptibility (CRO)
- Carbapenemase production carries major infection-control implications beyond individual patient treatment since these genes can often be transferred to other organisms

*CLSI M100, 36th ed.; Tamma PD, Simner PJ. J Clin Microbiol. 2018;56(11):e01140-18.*

# When Results Don't Match Expectations

A patient with a multidrug-resistant *Pseudomonas aeruginosa* bloodstream infection is being treated. **The formulary carbapenem tests resistant, but a closely related carbapenem in the same drug class tests susceptible on the panel.**

*Reflex question: “They're in the same class — can we just assume the untested one works too?”*

## The Teaching Point

- Agents within the same class can show discordant susceptibility results, especially in organisms with efflux-pump or permeability-based resistance mechanisms
- “Class representative” or equivalent-agent testing (using one drug's result to predict another's) is on a case-by-case basis precisely because it can be misleading in exactly this way
- Each agent on the panel must be tested and reported on its own merits — never assume activity for an untested drug based on a same-class relative unless specified in your CLSI or EUCAST guidance documents



## ● THE BIGGER PICTURE

# Antimicrobial Resistance (AMR): CDC vs. WHO Priority Frameworks

*Two bodies, two priority lists for the same global problem — different scope, same top threats.*

## CDC — Antibiotic Resistance Threats Report

*United States, 2019 (updated 2021–2022)*

### ● URGENT

- Carbapenem-resistant Enterobacterales (CRE)
- Carbapenem-resistant Acinetobacter
- Clostridioides difficile
- Drug-resistant Neisseria gonorrhoeae
- Candida auris

### ● SERIOUS

- ESBL-producing Enterobacterales
- MRSA · VRE
- Multidrug-resistant Pseudomonas aeruginosa
- Drug-resistant Candida (non-auris)

### ● CONCERNING

- Erythromycin-R group A Strep
- Clindamycin-R group B Strep

## WHO — Bacterial Priority Pathogens List

*Global, 2024 (BPPL)*

### ● CRITICAL PRIORITY

- Carbapenem-resistant Acinetobacter baumannii
- Carbapenem-resistant Enterobacterales
- 3rd-gen cephalosporin-resistant Enterobacterales
- Rifampicin-resistant Mycobacterium tuberculosis

### ● HIGH PRIORITY

- Fluoroquinolone-resistant Salmonella Typhi
- Fluoroquinolone-resistant Shigella spp.
- Drug-resistant Neisseria gonorrhoeae
- Multidrug-resistant Pseudomonas aeruginosa
- Methicillin-resistant Staphylococcus aureus (MRSA)

### ● MEDIUM PRIORITY

- Several resistant streptococci/enterococci

**Same core threats, different lens:** CRE and carbapenem-resistant Acinetobacter top both lists. CDC's U.S. list also covers fungi and C. difficile; WHO's bacteria-only BPPL is built to prioritize global antibiotic R&D — which is why MRSA and *N. gonorrhoeae* rank High for WHO but Urgent/Serious for CDC.



## ● THE BIGGER PICTURE

# The Antibiotic Pipeline Has Not Kept Pace

- Only about five genuinely novel antibiotic classes have reached the market since 2000 — most new approvals are variations on existing classes.
- FDA antibiotic approvals fell from roughly 19 in the early 1980s to single digits in several recent five-year windows, a trend widely described as the “antibiotic discovery void.”
- Economic factors — short treatment courses, stewardship-driven conservative use, and generic competition — make antibiotic development less commercially attractive than other therapeutic areas.

## Why This Makes AST More Important, Not Less

With fewer new agents entering practice, **preserving the effectiveness of existing antibiotics through accurate susceptibility testing and antimicrobial stewardship is one of the few tools we have to keep pace with resistance.** Every correctly performed AST result helps a clinician use the right drug — and avoid using drugs that don't need to be used at all.

*Innovative perspectives on the discovery of small molecule antibiotics. npj Antimicrob Resist. 2025;3:24. Current economic and regulatory challenges in developing antibiotics for Gram-negative bacteria. npj Antimicrob Resist. 2025.*



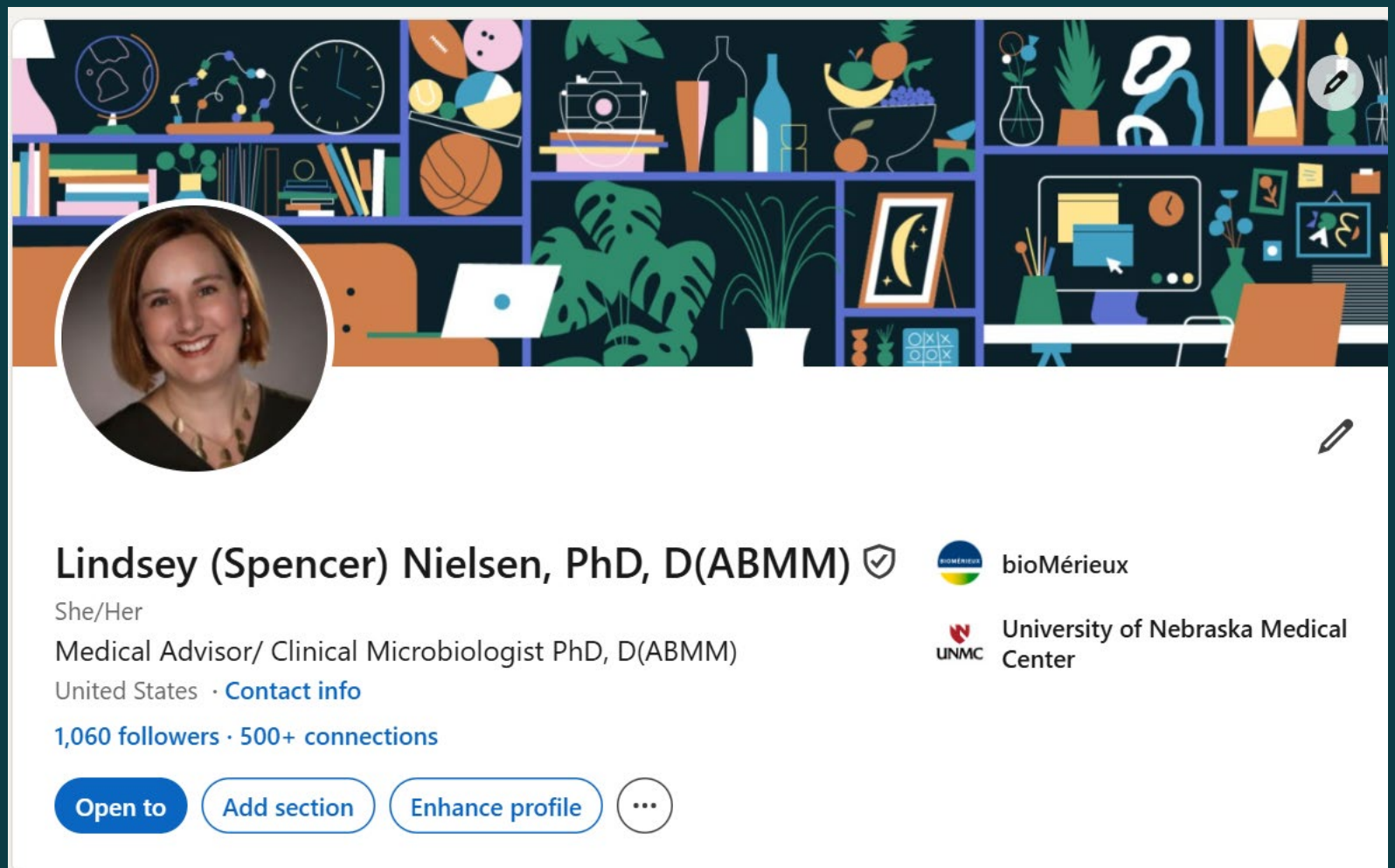
## ● REFERENCES

# Sources Cited

1. Fleming A. On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of *B. influenzae*. *Br J Exp Pathol*. 1929;10(3):226–236.
2. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clin Infect Dis*. 2009;49(11):1749–1755.
3. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 36th ed. CLSI supplement M100. CLSI; 2026.
4. CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests. CLSI standard M02. CLSI.
5. CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. CLSI standard M07. CLSI.
6. The European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters. Available at [eucastrg.org](http://eucastrg.org).
7. Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. *Clin Infect Dis*. 1998;26(1):1–10.
8. Swenson JM, Anderson KF, Lonsway DR, et al. Accuracy of cefoxitin disk diffusion testing for detection of *mecA*-mediated resistance in *Staphylococcus aureus*. *J Clin Microbiol*. 2005;43(8):3818–3823.
9. McConeghy KW, Bleasdale SC, Rodvold KA. The empirical combination of vancomycin and a beta-lactam for staphylococcal bacteremia. *Clin Infect Dis*. 2013;57(12):1760–1765.
10. Tamma PD, Simner PJ. Phenotypic detection of carbapenemase-producing organisms from clinical isolates. *J Clin Microbiol*. 2018;56(11):e01140-18.
11. van Belkum A, Burnham CD, Rossen JWA, et al. Innovative and rapid antimicrobial susceptibility testing systems. *Nat Rev Microbiol*. 2020;18(5):299–311.
12. Centers for Disease Control and Prevention. Antibiotic Resistance Threats in the United States, 2019. U.S. Department of Health and Human Services, CDC; 2019.
13. Centers for Disease Control and Prevention. Antimicrobial Resistance Threats in the United States, 2021–2022. U.S. Department of Health and Human Services, CDC; 2024.
14. Innovative perspectives on the discovery of small molecule antibiotics. *npj Antimicrob Resist*. 2025;3:24.

# Thank You

Questions?



<https://www.linkedin.com/in/lindsey-nielsen-micro/>