

Understanding & Applying Antimicrobial Susceptibility Data

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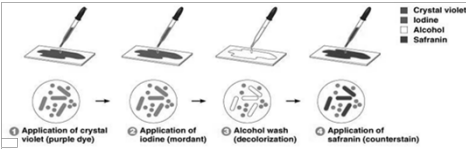
Objectives

- Identify the key components of an antimicrobial susceptibility report
- Differentiate between antimicrobial MIC and breakpoints
- Identify limitations of antimicrobial susceptibility data
- Incorporate antimicrobial susceptibility data in selection of an antimicrobial regimen

What is on the Susceptibility Report?

Blood Cult, Bact/Yst	Blood	Source
Specimen Descript	Blood	
Special Requests	None	
Gram Stain	Gram stain of blood culture medium: Gram negative rod	Gram stain
Culture	Escherichia coli This Gram negative organism is a multidrug resistant organism (MDRO). A person infected with a MDRO should be in appropriate precautionary isolation consistent with institutional recommendations.	Culture
Report Status	FINAL 03/23/10	Report Status
Blood Cult, Bact/Yst	Escherichia coli	MIC values and interpretation
Organism	Escherichia coli	
Method Type	MIC (Phoenix MIC)	
Amikacin	<=8 Susceptible	
Ampicillin	>16 Resistant	
Cefazolin	>16 Resistant	
Ciprofloxacin	>2 Resistant	
Gentamicin	<=2 Susceptible	
Meropenem	<=1 Susceptible	
Piperacillin/Tazo	>64/4 Susceptible	

Gram Stain

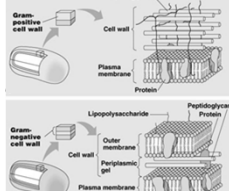


- Crystal violet
- Iodine
- Alcohol
- Safranin

① Application of crystal violet (purple dye) ② Application of iodine (mordant) ③ Alcohol wash (decolorization) ④ Application of safranin (counterstain)

"Gram Variable"


- Actinomyces
- Corynebacterium
- Mycobacterium
- Propionibacterium
- Bacillus
- Clostridium



Clinically Relevant Bacteria

Gram (+)


Cocci



Staphylococcus spp.
Streptococcus spp.
Enterococcus spp.

Anaerobes:
Peptococcus
Peptostreptococcus

Bacilli




C. Diphtheria
L. Monocytogenes
Bacillus spp.
Actinomycetes spp.

Anaerobes:
Clostridium spp.
Actinomyces spp.
Propionibacterium

Gram (-)


Cocci



Neisseria spp.
M. catarrhalis

Cocco-bacilli:
Hemophilus spp.
Bordetella pertussis

Bacilli



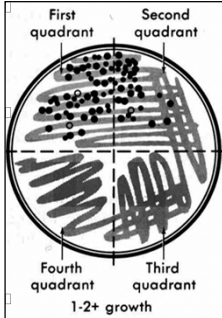
Enterobacteriaceae:
E. coli, Klebsiella, Proteus, Serratia, Enterobacter, Salmonella

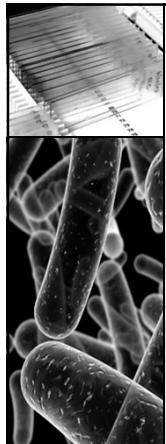
Pseudomonas

Anaerobes:
Bacteroides
Fusobacterium

Quantitative Interpretation

- "Rare" does not imply the pathogen is unusual
- Laboratory technicians visually quantify the number of organisms:
 - Rare (1+): < 1 cfu per field
 - Few (2+): 1-10 cfu
 - Moderate (3+): 11-25 cfu
 - Many (4+): > 25 cfu
- Depends on source of culture, organism



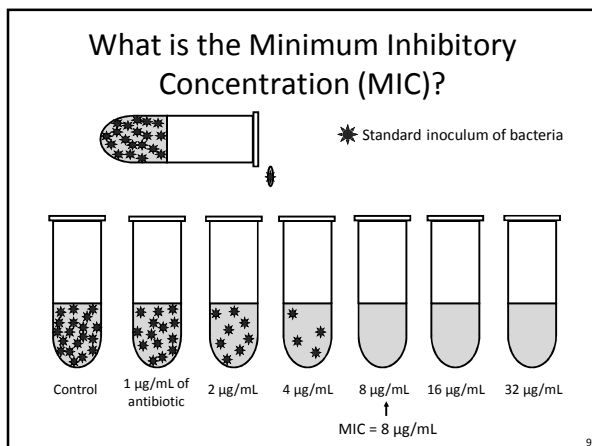


Methods for Determining MICs





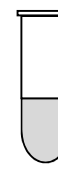
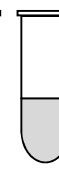
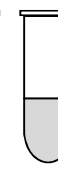
Broth Dilution Tests

- Prepare 2-fold (\log_2) dilutions of antibiotics in a liquid growth medium dispensed in test tubes
- Inoculate tubes with a standard bacterial suspension ($1-5 \times 10^5$ cfu/mL)
- Incubate at 35°C overnight
- Examine tubes for visible bacterial growth, evidenced by turbidity
- The lowest concentration of antibiotic that prevented growth represents the minimum inhibitory concentration (MIC)

What is the Minimum Inhibitory Concentration (MIC)?



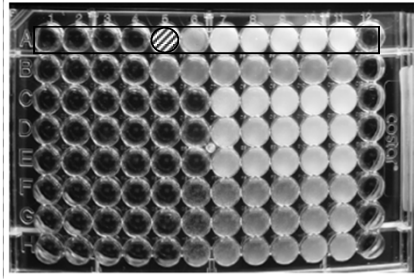
* Standard inoculum of bacteria

						
Control	1 µg/mL of antibiotic	2 µg/mL	4 µg/mL	8 µg/mL	16 µg/mL	32 µg/mL
				↑		
				MIC = 8 µg/mL		

Broth Microdilution Method

Each column (denoted 1-11) of wells contains a standard antibiotic concentration that doubles when moving right to left. Column 12 is a sterile control

Each row contains standard dilutions of eight bacterial organisms (denoted A-H)



10

Automated Systems: BD Phoenix

Identification and Susceptibility

NMIC/ID Panel Identifier Code	Phoenix AST Panels • Crain-Neigam							
	NMIC/ID 121	NMIC/ID 122	NMIC/ID 123	NMIC/ID 124	NMIC/ID 126	NMIC/ID 127	NMIC/ID 128	
NMIC/ID Catalog Number	448721	448722	448723	448724	448725	448727	448728	
NMIC Panel Identifier Code	NMIC 121	NMIC 122	NMIC 123	NMIC 124	NMIC 126	NMIC 127	NMIC 128	
NMIC Catalog Number	448672	448673	448674	448675	448677	448678		
Aminikacin	8-32	8-32	8-32	8-32	4-32	8-32	8-32	
Aminocyclitol	42-168	42-168	—	—	42-168	42-168	—	
Amphotericin	4-16	4-16	4-16	4-16	4-16	4-16	4-16	
Amphotericin/Biobam	—	—	165-168	165-168	—	165-168	165-168	
Amphotericin	2-16	2-16	2-16	2-16	2-16	2-16	2-16	
Cefazolin	2-16	2-16	2-16	2-16	2-16	2-16	2-16	
Cefepime	1-16	1-16	1-16	1-16	1-16	1-16	1-16	
Ceftazidime	4-32	4-32	—	—	—	—	—	
Ceftazidime	4-16	4-16	4-16	—	—	—	—	
Ceftazidime	0.5-2	0.5-16	0.5-16	0.5-16	0.5-16	0.5-16	0.5-16	
Ceftiofur	2-32	—	2-32	2-32	2-32	2-32	2-32	
Cefuroxime	4-16	4-16	4-16	—	—	—	—	
Ciprofloxacin	0.5-2	0.5-2	0.5-2	0.5-2	0.5-2	0.5-2	0.5-2	
Clotrimazole	0.5-4	0.5-4	—	—	—	—	—	
URA Confirmatory	YES	YES	YES	YES	YES	YES	YES	
Genesense	—	—	—	—	—	—	—	
Imipenem	1-8	1-8	1-8	—	1-8	1-8	—	
Linezolid	1-8	1-8	1-8	1-8	1-8	1-8	1-8	
Miconazole	1-8	1-8	1-8	1-8	—	1-8	1-8	
Miconazole	1-8	1-8	1-8	1-8	1-8	1-8	1-8	
Miconazole	1-8	1-8	1-8	1-8	1-8	1-8	1-8	
Nitrofur Antid	—	—	—	—	8-32	—	—	
Nitrofurantoin	16-64	16-64	16-64	16-64	16-64	16-64	16-64	
Nitrofurantoin	4-64	—	—	—	—	—	—	
Piperacillin	2-64	2-64	2-64	2-64	2-64	2-64	2-64	
Piperacillin/Tazobactam	2-8	2-8	2-8	2-8	—	—	—	
Tetracycline	2-8	2-8	2-8	2-8	—	—	—	
Tetracycline	2-8	2-8	2-8	2-8	2-8	2-8	2-8	
Trometamol	0.565-238	0.565-238	0.565-238	0.565-238	0.565-238	0.565-238	0.565-238	
Trometamol	0.565-238	0.565-238	0.565-238	0.565-238	0.565-238	0.565-238	0.565-238	

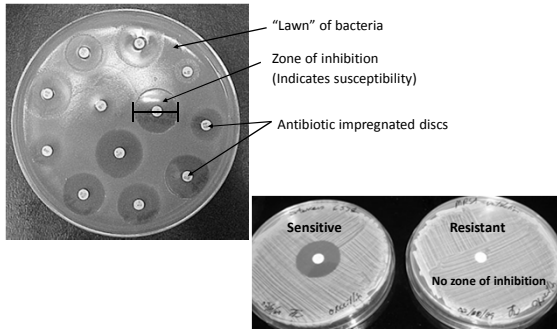
11

Disk Diffusion Test

- Developed as a practical alternative to the dilution tests
- The test agar plate is swabbed with a standard concentration of bacteria (“lawn”)
- Paper discs impregnated with antibiotic are placed on the “lawn”
- After overnight incubation, the diameter of the zone of inhibited growth around the disc is measured
 - The larger the zone, the more susceptible the organism to the antibiotic

12

Kirby-Bauer Agar Disk Diffusion



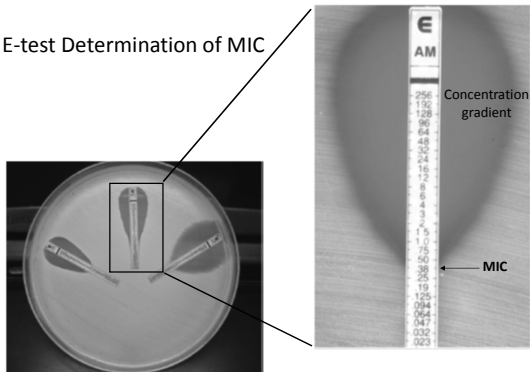
13

Antimicrobial Gradient Method

- Etest (bioMérieux AB BIODISK)
 - Cost \$2-3 per test
 - Manual method of exact MIC testing using a gradient technique
 - Usually good correlation between broth or agar dilution methods
 - Plastic strip is impregnated on the underside with a dried antibiotic concentration gradient and marked on the upper side with a concentration scale
 - MIC is determined by the intersection of the lower part of the ellipse shaped growth and test strip

14

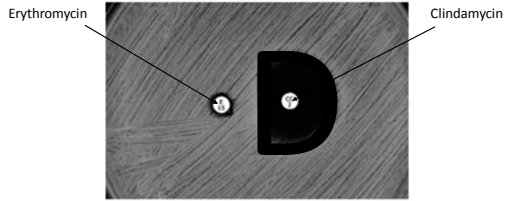
E-test Determination of MIC



Pharmacotherapy. 2009;29(11):1326-1343.

15

Specialty Test: Clindamycin D-Test



A positive D-test for inducible clindamycin resistance

Clin Infect Dis. 2005;40:280-285.

16

Detecting Resistance: Modified Hodge Test

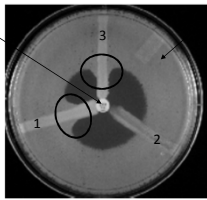
Carbapenemase Detection

10µg meropenem or ertapenem disc

(3) Clinical isolate

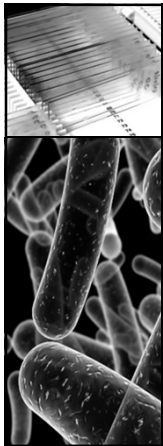
Mueller-Hinton agar plate with lawn of 1:10 dilution of *E. coli* (ATCC 25922)

(1) *K. pneumoniae* (ATCC BAA 1705), positive control



(2) *K. pneumoniae* (ATCC BAA 1706), negative control

17



MIC and Breakpoints

18

How are Breakpoints Set?

- Antibiotic breakpoints are critical values that correlate an MIC value to clinical outcomes
- Two stages for setting breakpoints
 - FDA initially sets breakpoints based on new drug application data, published in the package insert
 - CLSI correlates clinical outcomes to MIC values and may suggest revisions to established breakpoints

Clin Infect Dis. 2001;33(Suppl 3):S227-229.

19

Clinical and Laboratory Standards Institute (CLSI)

- NCCLS founded in 1968 for the purpose of developing voluntary consensus standards for clinical lab testing in the US
- Antimicrobial Susceptibility Testing (AST) Subcommittee
- Analyze 3 types of data for setting interpretive MIC breakpoints
 - Microbiological data
 - Pharmacokinetic and pharmacodynamic determinations
 - Review clinical and bacteriological response data collected during pre- and post-FDA drug approval

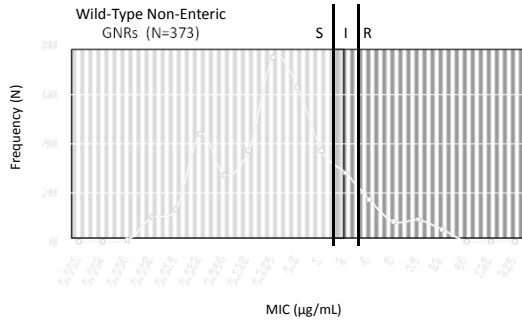
20

Factors Associated with Clinical Success/Failure

- Pathogen characteristics
 - Species
 - Mechanisms of resistance
- Infection characteristics
 - Site of infection
 - Presence of foreign material
 - Vascularization
- Patient characteristics
 - Age
 - Immune status
- Drug characteristics
 - Body weight
 - Comorbidities
- Drug characteristics
 - Dose & formulation
 - PK/PD

21

What is the Difference Between the MIC and the Breakpoint?



22

Why Revise Breakpoints?

- Original breakpoints may not reflect decreased

A recent inventory of antibacterial drug labels indicated that among more than 100 currently approved drug labels, over 70 contained breakpoints that were outdated.

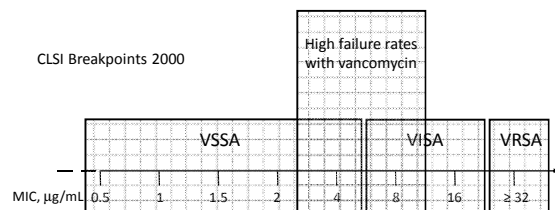
Infectious Diseases Society of America. Statement of the Infectious Diseases Society of America before the Food and Drug Administration part 15 hearing panel on antimicrobial resistance. April 28, 2008. Arlington, VA: Infectious Diseases Society of America, 2008.

- Development of new screening tests to detect resistant mechanisms (eg. *mecA*, ESBLs)

Clin Microbiol Infect. 2008;14(Suppl 1):169-174.
Clin Infect Dis. 2001;33(Suppl 3):S227-229.

23

Vancomycin Breakpoints for *Staphylococcus aureus* Infections



Clin Infect Dis. 2007;44:1208-15.

24

Site of Infection Matters!

Blood Cult, Bact/Yst	
Specimen Descript	Blood
Special Requests	None
Gram Stain	Gram positive cocci suggestive of Streptococci
Culture	
	Streptococcus pneumoniae
Report Status	FINAL 01/18/2010
Blood Cult, Bact/Yst	
Organism	Streptococcus pneumoniae
Method Type	MIC (Phoenix MIC)
Penicillin G	2 Intermediate (Performed by Etest)
Penicillin mening	2 Resistant (Performed by Etest)
Ceftriax (mening)	1 Intermediate
Vancomycin	<=0.25 Susceptible
Penicillin nonmen	2 Susceptible (Performed by Etest)
Ceftriaxone (non meni	1 Susceptible

28

True or False: Therapy should be chosen based on the drug with the lowest MIC value.

False

MICs are specific to each bug-drug combination
Higher values do not imply worse efficacy – if the interpretation is susceptible, then the antimicrobial should be considered a therapeutic option

29

Contaminants

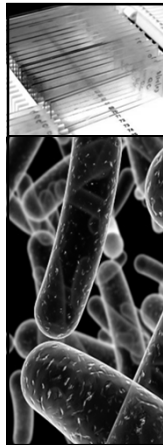
- | | |
|-------------------|--|
| Catheter Tip Cult | Central Venous Catheter |
| Specimen Descript | |
| Organism | Coagulase negative Staphylococcus |
| Special Requests | Multiple strains, all negative |
| Gram Stain | The number of Staphylococci not consistent with the catheter tip being the source of the infection. This assessment is only valid for cultures of surgically placed intravenous catheter tips. |
| Report Status | FINAL 03/20/2010 |
- Determining whether a Bug is a Contaminant or Not
 - Correlate clinical status to culture results (Temp, WBC)
 - If the bug grows in 2 out of 2 blood cultures, then it's probably not a contaminant
 - Sterile vs non-sterile sites)
 - Immune status
 - Common Contaminants
 - Bacillus spp.
 - Propionibacterium acnes
 - Alpha-hemolytic Streptococci

30

Limitations of Antimicrobial Susceptibility Testing

- Inoculum effect
 - MICs may be falsely low
 - May not accurately reflect resistant phenotypes
 - Can lead to therapeutic failures
- CLSI susceptibility guidelines not available for rare microorganisms
- Lack of MIC interpretations for new antimicrobial agents
- Breakpoints may not be current
- Detection of mechanisms of resistance are constantly evolving
- Need to be interpreted within the clinical context of the patient!

31



Clinical Applications: Things to Consider

32

ESBL Producing Organisms

Urine Culture	
Specimen Descript	Urine
Special Requests	None
Culture	>100,000 cfu/mL Escherichia coli This organism produces ESBL and meets the definition for MDR0.
Report Status	FINAL 08/20/10
Organism	>100,000 cfu/mL Escherichia coli
Method Type	MIC (Phoenix MIC)
Ampikacin	<=8 Susceptible
Ampicillin	>16 Resistant
Ampicillin/Sulbac	>16/8 Resistant
Aztreonam	<=2 Resistant
Cefazolin	>16 Resistant
Cefepime	<=1 Resistant
Ceftriaxone	32 Resistant
Ciprofloxacin	>2 Resistant
Gentamicin	<=2 Susceptible
Meropenem	<=1 Susceptible
Nitrofurantoin	<=16 Susceptible
Piperacillin/Tazo	16/4 Susceptible
Sulfameth/trimeth	>2/38 Resistant

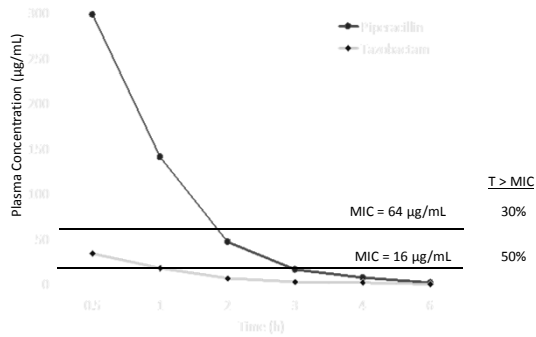
33

Which antibiotic would you recommend?

Aerobic Culture Swab	
Specimen Descript	Wound
Special Requests	None
Gram Stain	Very few Gram negative rods Very few epithelial cells seen
Culture	Heavy growth Pseudomonas aeruginosa Susceptibility testing to be confirmed
Report Status	PENDING
Aerobic Culture Swab	
Organism	Heavy growth Pseudomonas aeruginosa
Method Type	MIC (Phoenix MIC)
Amikacin	<=8 Susceptible
Cefepime	>16 Resistant
Ciprofloxacin	>2 Resistant
Gentamicin	>8 Resistant
Imipenem	>8 Resistant
Piperacillin/Tazo	64/4 Susceptible
Tobramycin	>8 Resistant

34

Piperacillin/tazobactam Kinetics



35

What antibiotic would you chose?

Sputum Culture + Gram Stain	
Specimen Descript	Sputum
Special Requests	None
Gram Stain	Few Gram positive cocci Numerous white blood cells seen Very few epithelial cells seen
Culture	Moderate growth Acinetobacter baumannii/calcoaceteticus complex Light growth mixed upper respiratory flora
Report Status	FINAL 08/11/10
Organism	Moderate growth Acinetobacter baumannii/calcoaceteticus complex
Method Type	MIC (Phoenix MIC)
Amikacin	<=8 Susceptible
Cefepime	2 Susceptible
Ciprofloxacin	<=0.5 Susceptible
Gentamicin	<=2 Susceptible

Ampicillin/sulbactam?

36

Class Substitutions

Blood Cult, Bact/Yst	
Specimen Descript	Blood
Special Requests	None
Gram Stain	Gram negative rod
Culture	Escherichia coli This Gram negative organism is a multidrug resistant organism (MDRO).
Report Status	FINAL 06/08/10
Organism	Escherichia coli
Method Type	MIC (Phoenix MIC)
Amikacin	<=8 Susceptible
Ampicillin	>16 Resistant
Ampicillin/sulbac	>16/8 Resistant
Aztreonam	<=2 Susceptible
Cefazolin	16 Intermediate
Ceftriaxone	<=2 Susceptible
Ciprofloxacin	2 Resistant
Gentamicin	<=2 Susceptible
Meropenem	<=1 Susceptible
Piperacillin/tazo	4/4 Susceptible
Sulfameth/trimeth	>2/38 Resistant

37

Clinical Practice Summary

- Site of infection
 - Penetration
 - Distribution
- Drug Selection
 - Empiric – refer to antibiograms
 - Drug susceptibility – de-escalate to narrowest spectrum antibiotic
- Patient Factors
 - Renal and hepatic dysfunction
 - Obesity, CF Patients, pediatrics, and geriatrics
 - Maximize pharmacokinetic/pharmacodynamic parameters
- Administration
 - Severe infections – intravenous vs. oral
 - Dosing frequency – compliance
 - Bioavailability

38

Questions?

It was on a short-cut through the hospital kitchens that Albert was first approached by a member of the Antibiotic Resistance.

http://www.lab-initio.com/sci_bio_microbiology.html

39

Coming Soon: Updated Breakpoints for Cephalosporins

EUCAST and CLSI felt existing breakpoints did allow for detection of important resistance mechanisms (ESBLs) and did not correlate well with clinical outcomes. The revised breakpoints are not meant to detect all ESBL-producing isolates of Enterobacteriaceae.

	Cefuroxime		Cefotaxime		Ceftriaxone		Ceftazidime		Cefepime	
	S	R	S	R	S	R	S	R	S	R
Current breakpoints	≤ 8	> 16	≤ 8	> 32	≤ 8	> 32	≤ 8	> 16	≤ 8	> 16
EUCAST	≤ 8	> 8	≤ 1	> 2	≤ 1	> 2	≤ 1	> 8	≤ 1	> 16
CLSI	≤ 8	> 8	≤ 1	> 2	≤ 1	> 2	≤ 4	> 8	≤ 8	> 16

Clin Microbiol Infect. 2008;14(Suppl 1):169-174.

40
