Diagnostic Antimicrobial Resistance Testing

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Disclosures

- Research Contracts:
  - BD Diagnostics, Accelerate Diagnostics, OpGen Inc., Affinity Biosensors, Qiagen Sciences Inc.
- Speaker's Bureau
  - GenMark Dx, BD Diagnostics
- Research Collaborators:
  - Ares Genetics, CosmosID, IDbyDNA, Illumina, Nanopore
- Consulting:
  - OpGen Inc., BD Diagnostics, Shionogi Inc., GeneCapture, Entasis, Merck & Co.
- CLSI AST Subcommittee voting member & member of the CAP Microbiology committee

Objectives

- Describe the various phenotypic and genotypic methods for the detection of bacterial antimicrobial resistance
- Provide examples of implementing AMR detection tests based on clinical needs
- List the resources available to implement tests for the detection of antimicrobial resistance for clinical and public health purposes

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Let's Rewind to March, 1942

Source: National Museum of American History

- Mrs. Anne Miller of New Haven, Connecticut was near death due to a bloodstream infection
  - Administered an experimental drug: penicillin
  - A drug that was discovered by Alexander Flemming in 1928
- 1st person to be saved by antibiotics
- Widely used in World Word II for surgical and wound infections

www.nytimes.com/1999/06/09/us/anne-miller-90-first-patient-who-was-saved-by-penicillin.html

1960's: "[It] is time to close the book on infectious diseases and declare the war against pestilence won"
– William H Stewart (US Surgeon General)

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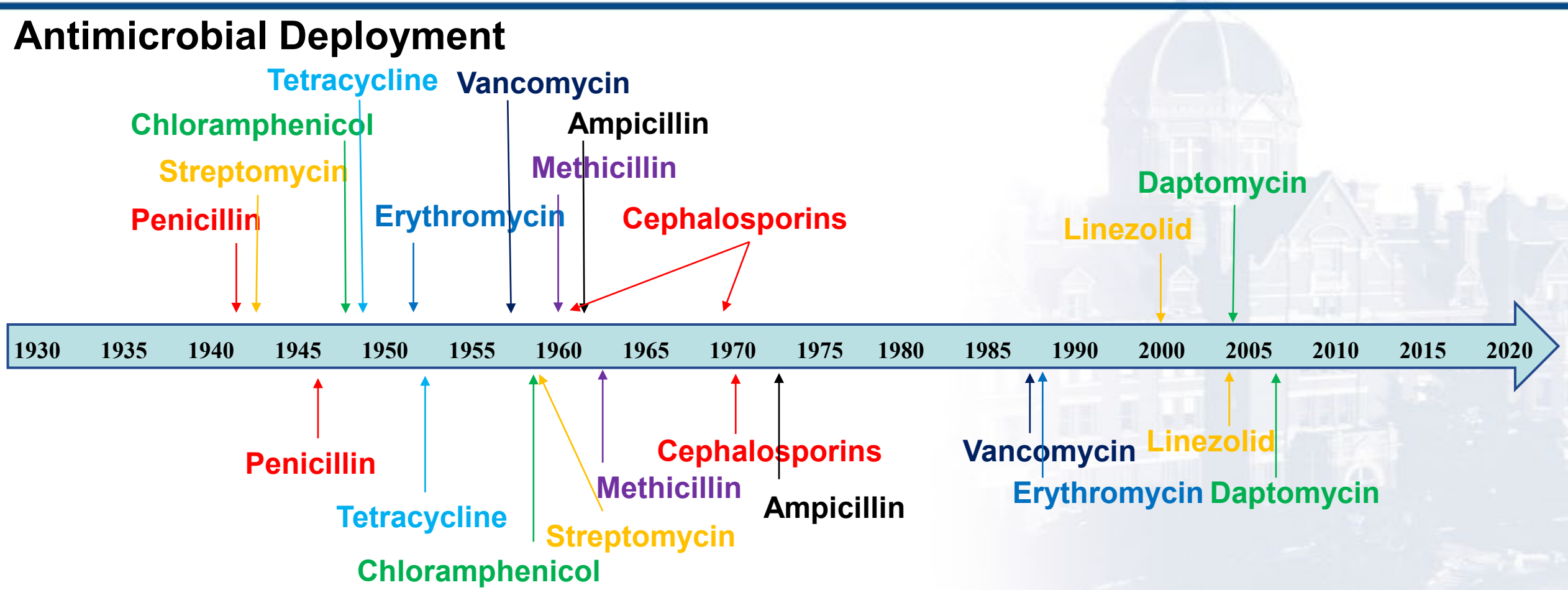
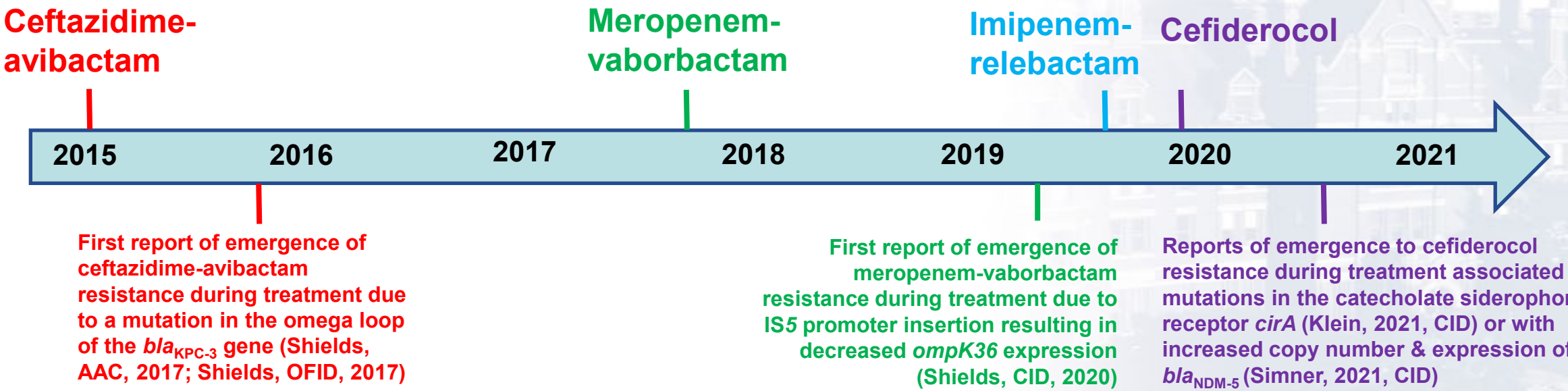
The Bugs are Always Smarter Than the Drugs



The Bugs are Always Smarter Than the Drugs

Antimicrobial Deployment



Antimicrobial Resistance Detected

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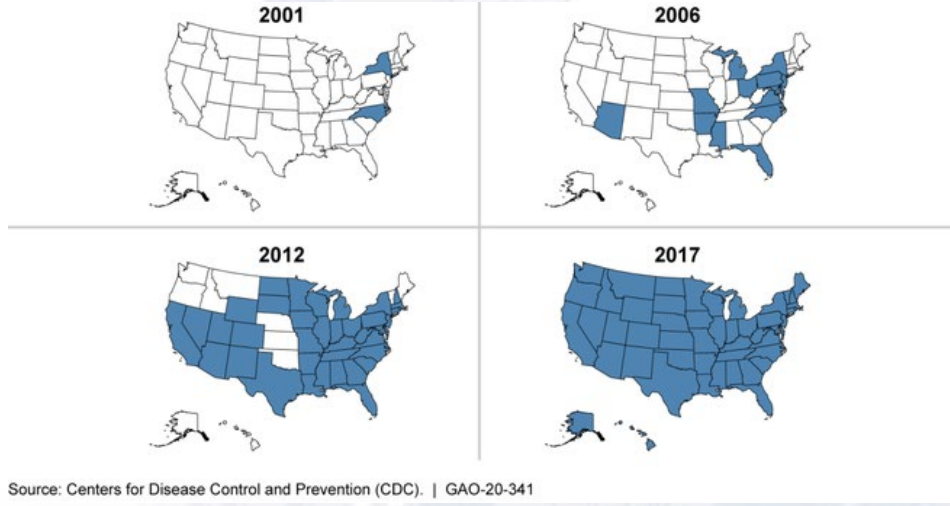
The Threat of Antimicrobial Resistance

- One of the biggest global public health threats
  - Recognized by many international bodies
- Leading cause of death
  - Highest burden in resource limited settings
- Precise magnitude is not well understood
  - 2019: 4.95 million deaths associated with AMR, including 1.27 million deaths attributed to bacterial AMR
- Global collective action is required
  - Improve Global Surveillance for Antimicrobial Resistance
  - Promote New, Rapid Diagnostics to Optimize Antibiotic Use



Tracking the spread of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Enterobacterales* a type of carbapenem-resistant *Enterobacterales* (CRE) by the CDC.

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Antimicrobial Resistance Collaborators, Lancet, 2022. O'Neil, The Review on Antimicrobial Resistance, 2016; Burnham et al, ICHE, 2019; https://www.gao.gov/products/GAO-20-341.

The Post-Antibiotic Era

- “Stop referring to a coming post-antibiotic era – it’s already here”
  – Robert Redfield, M.D.

www.cdc.gov/DrugResistance/Biggest-Threats.html

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We Are Facing It in the Microbiology Laboratory

Susceptibility

	Klebsiella pneumoniae	
	MIC	BP
Amikacin		>128 ug/mL
Ampicillin	>16 ug/mL R	
Ampicillin + Sulbactam	>16/8 ug/mL R	
Aztreonam	>16 ug/mL R	
Cefazolin	>16 ug/mL R	
Cefepime		>16 ug/mL
Cefoxitin	>16 ug/mL R	
Ceftazidime		
Ceftriaxone		
Ciprofloxacin		
Ertapenem		
Gentamicin		
Meropenem		
Piperacillin + Tazobactam		
Tetracycline		
Tigecycline		
Tobramycin		
Trimethoprim + Sulfamethoxazole		

Susceptibility

Amikacin		
Amoxicillin-Clavulanate		
Ampicillin		
Ampicillin-Sulbactam		
Aztreonam		
Cefazolin		
Cefepime		
Cefiderocol		
Ceftazidime		
Ceftazidime-Avibactam		
Ceftriaxone		
Cefuroxime	>16 ug/mL R	
Ciprofloxacin	>2 ug/mL R	
Ertapenem	>2 ug/mL R	
Fosfomycin		S
Gentamicin	<=2 ug/mL S	
Meropenem	>8 ug/mL R	
Meropenem-Vaborbactam	>16/8 ug/mL R	
Nitrofurantoin	32 ug/mL S	
Piperacillin-Tazobactam	>64/4 ug/mL R	
Tetracycline	>8 ug/mL R	
Tigecycline	>8 ug/mL R	
Tobramycin	>8 ug/mL R	
Trimethoprim-Sulfamethoxazole	>2/38 ug/mL R	

Susceptibility

	Pseudomonas aeruginosa		
	MIC	BP	KB
Amikacin	>32 ug/mL R		
Ampicillin			
Aztreonam	>16 ug/mL R		
Cefepime	>16 ug/mL R		
Cefiderocol			R
Ceftazidime	>16 ug/mL R		
Ceftazidime-Avibactam	>8/4 ug/mL R		
Ceftolozane-Tazobactam	>8/4 ug/mL R		
Ciprofloxacin	>2 ug/mL R		
Clindamycin			
Colistin		<=1 ug/mL I [1]	
Daptomycin			
Erythromycin			
Gentamicin	>8 ug/mL R		
Imipenem-relebactam			R
Linezolid			
Meropenem	>8 ug/mL R		
Oxacillin			
Piperacillin-Tazobactam	>64/4 ug/mL R		
Quinupristin-Dalfopristin			
Tetracycline			
Tobramycin	>8 ug/mL R		

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WHAT CAN WE DO TO TACKLE AMR IN LABORATORY MEDICINE?

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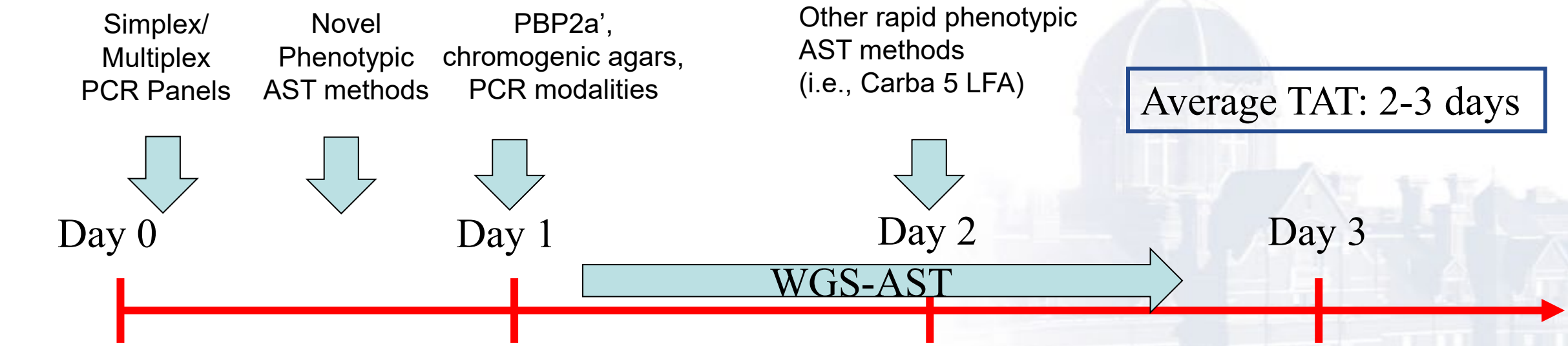
Current Paradigm for ID & AST



Empiric Treatment | **Narrowed Treatment** | **Targeted Treatment**

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AST: antimicrobial susceptibility testing; ID: identification; whole genome sequencing-AST: WGS to predict AST

Breakpoints = Stop Light Approach to Guide Therapy

- **The most critical step in AST involves interpretation of results!**
  - **Susceptible (S):** Isolates are inhibited by usually achievable concentrations of drug and dosing for that particular site of infection
    - **Resulting in likely clinical efficacy**
  - **Susceptible-Dose Dependent (SDD):** MIC/zone diameter for the isolate is dependent on the dosing regimen that is used in this patient
    - **Increasing the dose (if PK/PD parameters allow) increases the likelihood of clinical efficacy**
  - **Intermediate (I):** MICs/zone diameters for that isolate approach the usually achievable concentration of drug
    - Addresses ambiguity in testing methods
    - **Response may be lower than for susceptible isolates**
  - **Resistant (R):** Isolates are not inhibited by usually achievable concentrations of drug
    - **Resulting in a likely unfavorable outcome**

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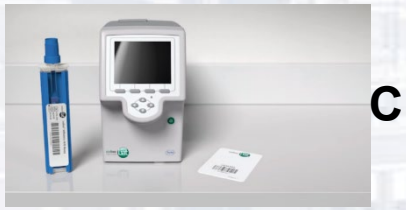
Many New Toys in The Clinical Microbiology Laboratory

Proteomic Based ID: MALDI-TOF MS

Moderately Complex Closed Systems- Sample- to-Answer devices
Syndromic Multiplex Molecular Panels

Sophisticated Advanced NGS Technologies



CLIA waived PCR POC devices

Total Laboratory Automation

Rapid Phenotypic AMR or AST Methods

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Diagnostic Methods to Detect AMR

- Phenotypic
  - Chromogenic media
  - Assays built into commercial AST panels
  - Assays performed from isolates
- Immunologic
  - Performed from cultured isolates
    - E.g. Carba 5 lateral flow assay
- Molecular Methods
  - Cultured isolates or directly from specimen

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Available and Forthcoming Molecular Panels

Source	Test	AMR genes	TAT (hr)	FDA Status
Whole blood	T2 Resistance	*mecA/C, vanA/B,* bla_{CTX-M}, bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, $bla_{OXA-23/OXA-48-like}$, bla_{CMY}, bla_{DHA}	3-5	
+ Blood Cultures	Xpert MRSA/SA BC	*mecA*	1	✓
	Biofire BC-IDII	*mecA, vanA/B,* bla_{CTX-M}, bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{OXA}	1	✓
	Verigene BC-GP & BC-GN	*mecA, vanA/B,* bla_{CTX-M}, bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{OXA}	2.5	✓
	Genmark BCID-GP & -GN	*mecA/C, vanA/B,* bla_{CTX-M}, bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, $bla_{OXA-23/OXA-48-like}$	1.5	✓
Respiratory	Biofire Panel Pneumonia	*mecA/C, MREJ vanA/B,* bla_{CTX-M}, bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{OXA-48}	1	✓
	Curetis Unyvero	Expanded panel	4-5	✓
Isolates	Xpert Carba-R	bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, $bla_{OXA-48-like}$		✓
	OpGen Acuitas AMR panel	Expanded panel		✓
	WGS	Comprehensive (known AMR)		???

Remember: It Does not Need to Be Expensive Tools

- Reporting comments
- AST Suppression rules
- AST Cascade reporting
- Less expensive phenotypic modalities

Enterobacter cloacae complex, *Klebsiella* (formerly *Enterobacter*) *aerogenes* and *Citrobacter freundii* complex may quickly develop resistance during therapy with 3rd-generation cephalosporins (e.g., ceftriaxone, ceftazidime) due to production of AmpC beta-lactamases. This does not apply to cefepime. Refer to the JHH/BMC Antibiotic Guidelines for Antibiotic Use Apps for adults or the Pediatric Antibiotic Guidelines for children for further guidance.

Susceptibility

	Klebsiella (Enterobacter) aerogenes MIC	Klebsiella pneumoniae complex MIC
Amikacin	<=8 ug/mL S	<=8 ug/mL S
Ampicillin	>16 ug/mL R	>16 ug/mL R
Ampicillin-Sulbactam	>16/8 ug/mL R	8/4 ug/mL S
Aztreonam	<=2 ug/mL S	<=2 ug/mL S
Cefazolin	>16 ug/mL R	2 ug/mL S
Cefepime	<=1 ug/mL S	<=1 ug/mL S
Cefoxitin	>16 ug/mL R	<=4 ug/mL S
Ceftazidime		<=2 ug/mL S
Ceftriaxone		<=1 ug/mL S
Ciprofloxacin	<=0.25 ug/mL S	<=0.25 ug/mL S
Gentamicin	<=2 ug/mL S	<=2 ug/mL S
Meropenem	<=0.5 ug/mL S	<=0.5 ug/mL S
Piperacillin-Tazobactam	4/4 ug/mL S	4/4 ug/mL S
Tobramycin	<=2 ug/mL S	<=2 ug/mL S
Trimethoprim-Sulfamethoxazole	<=0.5/9.5 u... S	<=0.5/9.5 u... S

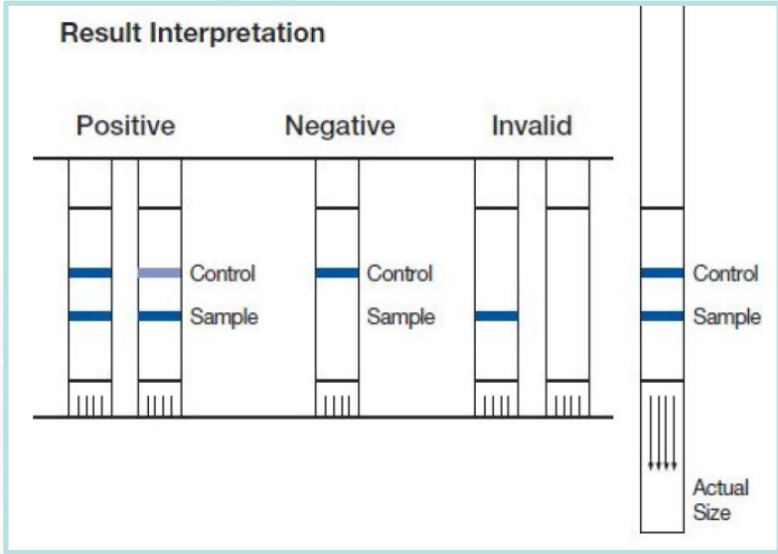
Presented with a Clinical Need…

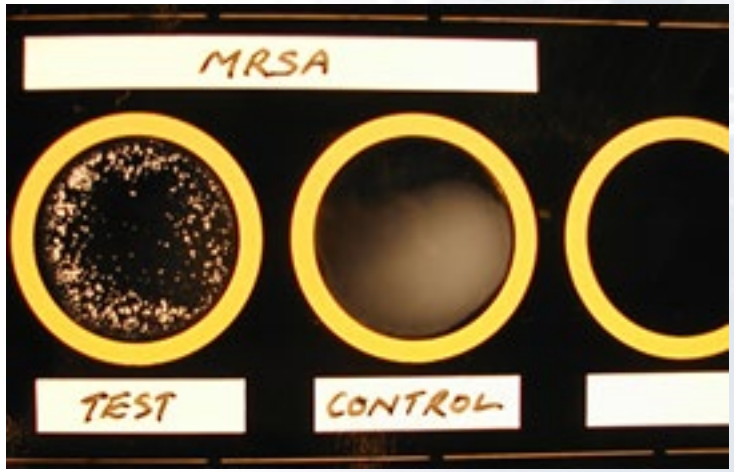
- 40-50% of *S. aureus* encountered at our institution is methicillin-resistant *S. aureus* (MRSA)
- Has led to empiric treatment of *S. aureus* infections with vancomycin
  - Waiting an additional day after ID to de-escalate therapy if MSSA
  - Prevents discharge
- Antimicrobial Stewardship wanted a method to rapidly detect MSSA earlier – *mecA* PCR

Trienski et al, Am J Health-Syst Pharm, 2013.

What about Phenotypic Methods? Penicillin-Binding Protein 2a (PBP2a)

- Rapid phenotypic method to detect the altered PBP2a encoded by *mecA* from cultured *S. aureus* isolates
  - PBP2a SA Culture Colony Test (Abbott Diagnostics)
    - Lateral flow assay
    - *S. aureus* (no induction)
  - PBP2a Latex agglutination assay (Thermo Fisher)
    - *S. aureus* (no induction) or CoNS (induction required)





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Laboratory Implications

- Costs: PCR vs Rapid Phenotypic Assay
  - PBP2a assay was cost-effective
- Workflow: How and when to perform?
  - Easily incorporated into the techs daily workflow
  - All sources (except urine and blood)
  - Batch twice daily
  - Perform on new *S. aureus* isolates (repeat after 30 days)
- Resources: Is additional staff required? Instrumentation?

PBP2a Reporting

Bacterial Culture+Smear, Aer/Ana, Misc

Lab Status: Final result

Specimen Information: Tissue from Left Lower

Gram Stain --

Result:

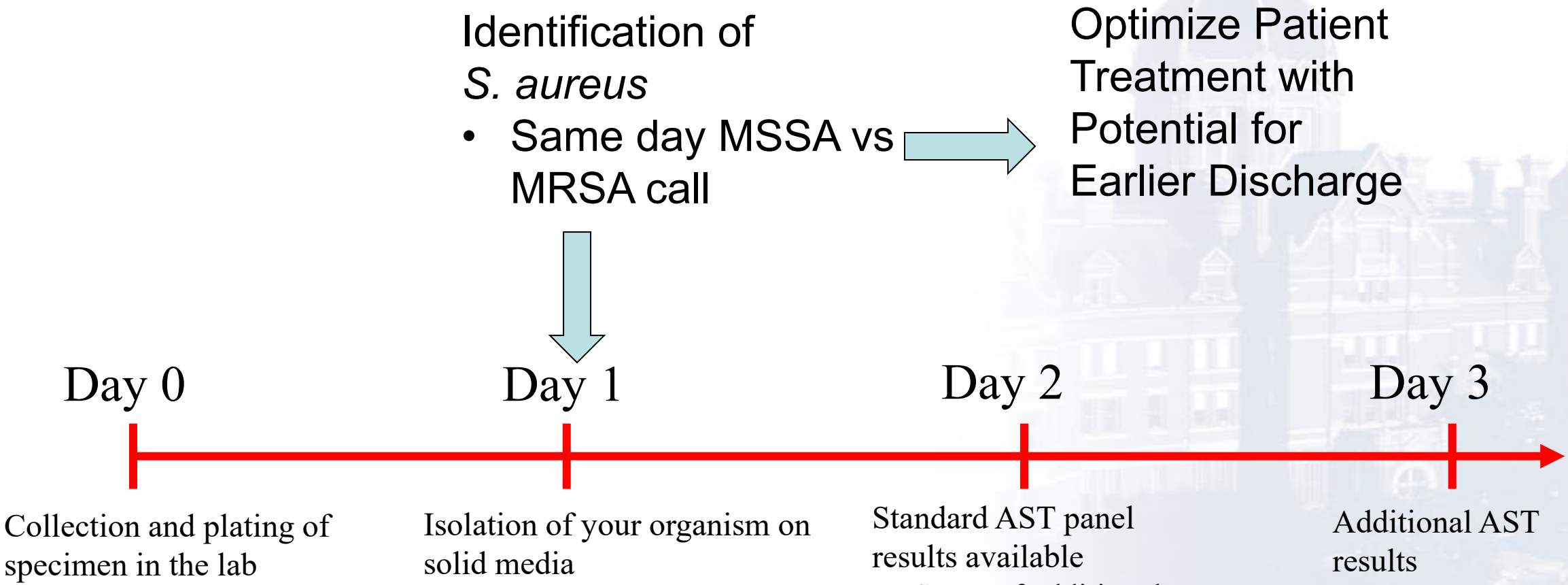
No Polymorphonuclear Leukocytes Seen

No organisms seen.

Aerobic/Anaerobic Misc Culture **Critical action value called to and read**

Methicillin resistant Staphylococcus aureus
In enrichment broth;
Staphylococcal isolates that are resistant to oxacillin should not be treated with penicillins, beta-lactam/beta-lactamase inhibitor combinations, carbapenems and cephalosporins except cephalosporins with
anti-MRSA activity.
Methicillin resistant by penicillin binding protein 2a (PBP2a)
lateral flow assay.
(HH)

Clinical Implications: A success story



Trienski et al, Am J Health-Syst Pharm, 2013.

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Presented with a Clinical Need…

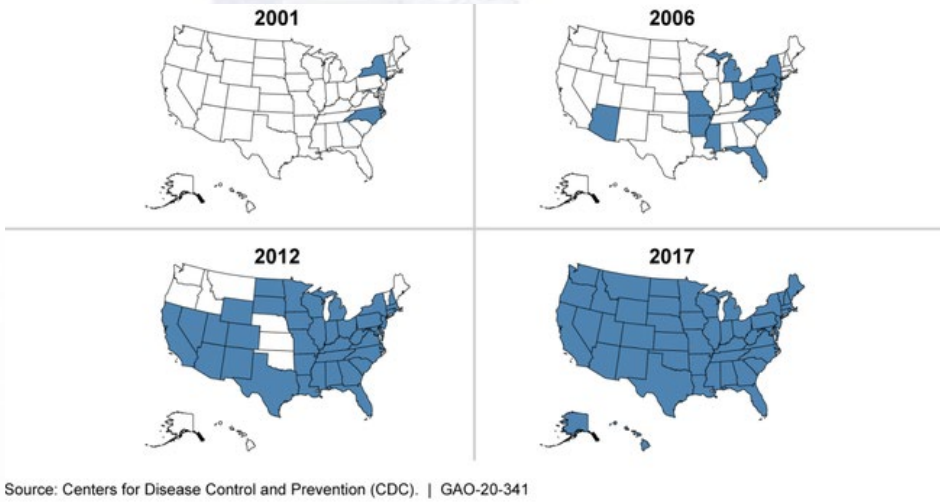
- Infection control: Identify carbapenemase-producing Enterobacterales (CPE) encountered in our facility for infection control measures
  - If it is a carbapenem-resistant Enterobacterales (CRE) and not a CPE→ remove patient from isolation

- Antimicrobial Stewardship: Is this a CPE? Do we know what the genotypic mechanism of carbapenem resistance is?
  - Can we use ceftazidime-avibactam, meropenem-vaborbactam?

Carbapenemases: A Triple Threat

- Enzymes that can hydrolyze all beta-lactams, including the carbapenems
  - The big 5: KPC, NDM, OXA-48, VIM & IMP

- Triple threat
  1. Increasing in prevalence globally – cause high morbidity and mortality – up to 60%
     - 5x greater risk of decreased survival for CP-CRE vs Non-CP-CRE
  2. Highly mobile – harbored on plasmids and flanked by insertion sequences or within a transposon
  3. Multidrug-resistant (MDR)



Tracking the spread of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Enterobacterales* a type of carbapenem-resistant *Enterobacterales* (CRE) by the CDC.

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Ben-David et al, Clin Microbiol Infect, 2012. **18**(1): p. 54-60. Patel et al, Infect Control Hosp Epidemiol, 2008. **29**(12): p. 1099-106. Tamma, CID, 2016.

Novel Agents Have Specific Niches

Agent (*US FDA approved)	KPCs	NDMs	OXA-48-like	Carbapenem-resistant *P. aeruginosa*	Carbapenem-resistant *A. baumannii*	*S. maltophilia*
Aztreonam-avibactam						
Cefiderocol*						
Ceftazidime-avibactam*						
Cefotolozane-tazobactam*						
Eravacycline*						
Imipenem-relebactam*						
Meropenem-vaborbactam*						
Plazomicin*						

Always confirm activity with antimicrobial susceptibility testing!

No activity | **Some Activity** | **Good activity**

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Why Is the Mechanism of AMR Important?

- Treatment guidance based on whether mechanism testing is performed or not
- Recommended treatment will differ based on the mechanisms mediating resistance

AMR	Recommended Treatment
ESBL	Meropenem
AmpC	Cefepime
Carbapenemase	≠ based on genotype

Source of Infection	Preferred Treatment	Alternative Treatment if First-line Options not Available or Tolerated
Cystitis	Ciprofloxacin, levofloxacin, trimethoprim-sulfamethoxazole, nitrofurantoin, or a single dose of an aminoglycoside	Ceftazidime-avibactam, meropenem-vaborbactam, imipenem-cilastatin-relebactam, and cefiderocol
	Meropenem[a] (standard infusion): only if ertapenem-resistant, meropenem-susceptible, AND carbapenemase testing results are either not available or negative	Colistin (when no alternative options are available)
Pyelonephritis or complicated urinary tract infection[b]	Ceftazidime-avibactam, meropenem-vaborbactam, imipenem-cilastatin-relebactam, and cefiderocol	Once-daily aminoglycosides
	Meropenem[a] (extended-infusion): only if ertapenem-resistant, meropenem-susceptible, AND carbapenemase testing results are either not available or negative	
Infections outside of the urinary tract Resistant to ertapenem, susceptible to meropenem, AND carbapenemase testing results are either not available or negative	Meropenem[a] (extended-infusion)	Ceftazidime-avibactam
Infections outside of the urinary tract	Ceftazidime-avibactam, meropenem-vaborbactam, and imipenem-cilastatin-relebactam	Cefiderocol
Resistant to ertapenem, resistant to meropenem, AND carbapenemase testing results are either not available or negative		Tigecycline, eravacycline (generally limited to intra-abdominal infections)
Klebsiella pneumoniae carbapenemases identified (or carbapenemase positive but identify of carbapenemase unknown[c])	Ceftazidime-avibactam, meropenem-vaborbactam, imipenem-cilastatin-relebactam	Cefiderocol
		Tigecycline, eravacycline (generally limited to intra-abdominal infections)
Metallo-β-lactamase (ie, NDM, VIM, IMP) carbapenemase identified	Ceftazidime-avibactam + aztreonam, cefiderocol	Tigecycline, eravacycline (generally limited to intra-abdominal infections)
OXA-48-like carbapenemase identified	Ceftazidime-avibactam	Cefiderocol
		Tigecycline, eravacycline (generally limited to intra-abdominal infections)

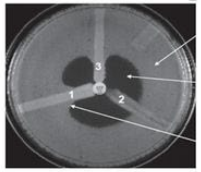
Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. Clin Infect Dis. 2020 Oct 27:ciaa1478. doi: 10.1093/cid/ciaa1478. Online ahead of print.Clin Infect Dis. 2020. PMID: 33106864

Methods to Detection CP-CRO

Phenotypic Detection Methods

Modified Hodge Test

A.



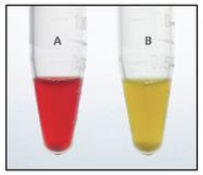
A lawn of carbapenem susceptible *E. coli* ATCC 25922

Zone of inhibition of *E. coli* ATCC 25922 by ertapenem

Indentation of *E. coli* ATCC 25922 growth (clover leaf appearance) around the streak line of the carbapenemase-producing *K. pneumoniae* ATCC BAA-1705.

Carba NP and variants

B.



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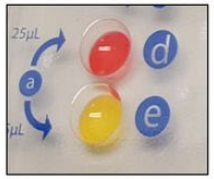

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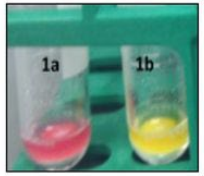
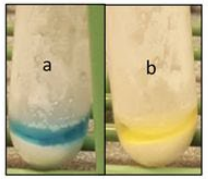


E.



Modified Carbapenem Inactivation Method (mCIM) & EDTA- mCIM (eCIM)

F.

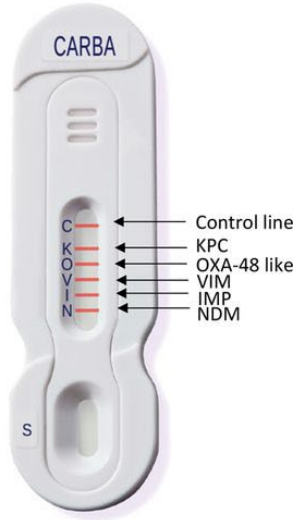
G.

H.

I.

Lateral Flow Immunoassay

J.



Molecular Detection Methods

Pranita D. Tamma, and Patricia J. Simner J. Clin. Microbiol. 2018; doi:10.1128/JCM.01140-18

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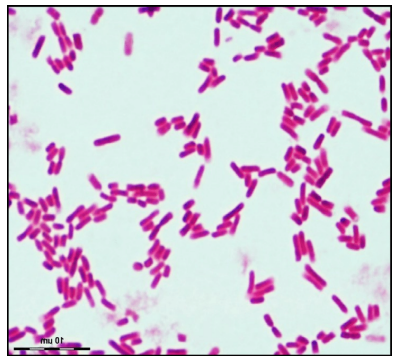
Different Approaches Based on Source

Blood culture bottle signals positive



Gram stain: CAV & gram- negative organism

If 1st + bottle from a patient: run the Gram-negative panel – repeat only after 7 days

Reported within 4hrs

Any organism above with resistance marker (s) listed below		
CTX-M (ESBL)	• Meropenem 1 g IV Q8H	Consult ID/ASP
KPC, OXA (CP-CRO)	• Ceftazidime/avibactam 2.5 g IV Q8H and Consult ID/ASP	Consult ID/ASP
IMP, NDM, VIM (CP-CRO)	• Cefiderocol 2 g IV Q8H and Consult ID/ASP	Consult ID/ASP

ePlex BCID-GN Panel	
Acinetobacter baumannii	
Bacteroides fragilis	
Citrobacter	
Cronobacter sakazakii	
Enterobacter (non-*cloacae* complex)	
Enterobacter cloacae complex	
Escherichia coli	
Fusobacterium necrophorum	
Fusobacterium nucleatum	
Haemophilus influenzae	
Klebsiella oxytoca	
Klebsiella pneumoniae group	
Morganella morganii	
Neisseria meningitidis	
Proteus	
Proteus mirabilis	
Pseudomonas aeruginosa	
Salmonella	
Serratia	
Serratia marcescens	
Stenotrophomonas maltophilia	
CTX-M	NDM
IMP	OXA
KPC	VIM
Pan *Candidae*	
Pan Gram-Positive	

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Our Algorithm at JHH for CRE

Susceptibility

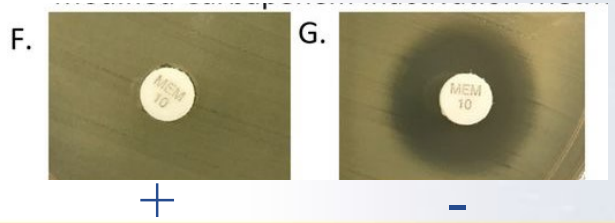
	Klebsiella pneumoniae			
	MIC		BP	
Amikacin			>128 ug/mL	R
Ampicillin	>16 ug/mL	R		
Ampicillin + Sulbactam	>16/8 ug/mL	R		
Aztreonam	>16 ug/mL	R		
Cefazolin	>16 ug/mL	R		
Cefepime			>16 ug/mL	R
Cefoxitin	>16 ug/mL	R		
Ceftazidime	>16 ug/mL	R		
Ceftriaxone	>32 ug/mL	R		
Ciprofloxacin	>2 ug/mL	R		
Ertapenem	>1 ug/mL	R		
Gentamicin	>8 ug/mL	R		
Meropenem	>8 ug/mL	R		
Piperacillin + Tazobactam	>64/4 ug/mL	R		
Tetracycline	>8 ug/mL	R		
Tigecycline			2 ug/mL	S
Tobramycin	>8 ug/mL	R		
Trimethoprim + Sulfamethoxazole	>2/38 ug/mL	R		

CRE – Cascade Protocol

Detection of carbapenemase producers

- Mono-ertapenem I/R -> Perform mCIM on CRE → if positive perform CARBA 5
- I/R to both ertapenem & meropenem -> Perform the CARBA 5 LFA same day

Phenotypic detection of carbapenemase production



10,000 CFU/mL ** *Klebsiella pneumoniae complex (Carbapenemase producer)*
Patient results disclosed to Maryland and/or
District of Columbia DOH.
Patient results disclosed to Maryland and/or

If mCIM positive – setup the CARBA 5 LFA

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Detection of Carbapenemase Producers - Clinical Implications

- Helps inform infection control strategies
  - Electronic Medical Record Flags based on reporting
  - Two-tiered response: Non-CP-CRE are not placed on contact precautions

- Helps with treatment based decisions
  - Work with antimicrobial stewardship to create cascade reporting rules, set-up or approve send-out of additional AST

Cascade Reporting Guidelines for MDRO Created With ASP

Organism	Cascade Rule	Agents Released or Setup Upon Cascade	Additional Agents Available Upon Request
Enterobacterales	I/R to ertapenem or meropenem	On panel agents: C-A M-V Tigecycline	On panel agents: Levofloxacin, ceftaroline, moxifloxacin, minocycline, C-T Additional Setup: Imipenem Colistin, I-R, cefiderocol for CRE
Pseudomonas aeruginosa	I/R to cefepime AND meropenem AND piperacillin-tazobactam	On-panel agents: C-T, C-A Setup and report: Colistin, I-R, cefiderocol	On panel agents: Levofloxacin Requires additional Setup: Imipenem
Acinetobacter baumannii complex	I/R to cefepime AND meropenem and piperacillin-tazobactam	Setup and report: Colistin Cefiderocol	On panel agents: Levofloxacin, tigecycline Requires additional Setup: Imipenem
Stenotrophomonas maltophilia	I/R to ceftazidime AND trimethoprim-sulfa AND levofloxacin AND minocycline	Setup and report: Cefiderocol	On panel agents: Cefepime, tigecycline Requires additional Setup: Colistin

Table 1A

M100 33rd Ed.

Table 1A: Enterobacterales (not including *Salmonella/Shigella*)[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[e] or Ceftriaxone[e]	Cefepime[f]		
	Ertapenem Imipenem Meropenem	Cefiderocol Ceftazidime-avibactam Imipenem-relebactam Meropenem-vaborbactam	
Amoxicillin-clavulanate Ampicillin-sulbactam Piperacillin-tazobactam			
Gentamicin	Tobramycin Amikacin	Plazomicin	
Ciprofloxacin Levofloxacin			
Trimethoprim-sulfamethoxazole			
	Cefotetan Cefoxitin		
	Tetracycline[b]		
			Aztreonam
			Ceftaroline[e]
			Ceftazidime[e]
			Ceftolozane-tazobactam
Urine only			
Cefazolin (surrogate for uUTI)[c]			
Nitrofurantoin			
		Fosfomycin[d] (*Escherichia coli*)	

Abbreviations. MDROs, multi-drug resistant organisms; uUTI, uncomplicated urinary tract infection

Reporting Tiers & Cascade Reporting Between Tiers

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HOW WOULD YOU VERIFY/VALIDATE A METHOD TO DETECT AMR?

Validation/Verification Plan

	Validation	Verification	Acceptance Criteria
	Performed to **establish** the performance characteristics	Performed to **verify** the performance characteristics	NA
Precision	Inter- (3 samples over 3 days) & Intra-assay (3 samples run 3 times on the same day) reproducibility	Inter- & Intra-assay reproducibility	≥ 95% precision
Accuracy	≥20 positive, 10 negative	20 positive, 10 negative	≥ 90% percent positive agreement (i.e., sensitivity) ≥ 90% percent negative agreement (i.e., specificity)
Reportable range	Not applicable (NA)	NA	Does not apply to qualitative assays
Reference range	Usually a negative result	Usually a negative result	Usually a negative result
Analytical sensitivity	Serial dilutions of samples with known concentrations in triplicate	NA	LOD is the lowest concentration where all 3 replicates are detected
Analytical specificity	A panel of potentially interfering or cross-reacting AMR genes	NA	Detection of only the intended targets

2x2 Contingency Table & Accuracy Calculations

Table 1. 2 x 2 Contingency Table

Comparator/Reference Method

Method Being Evaluated

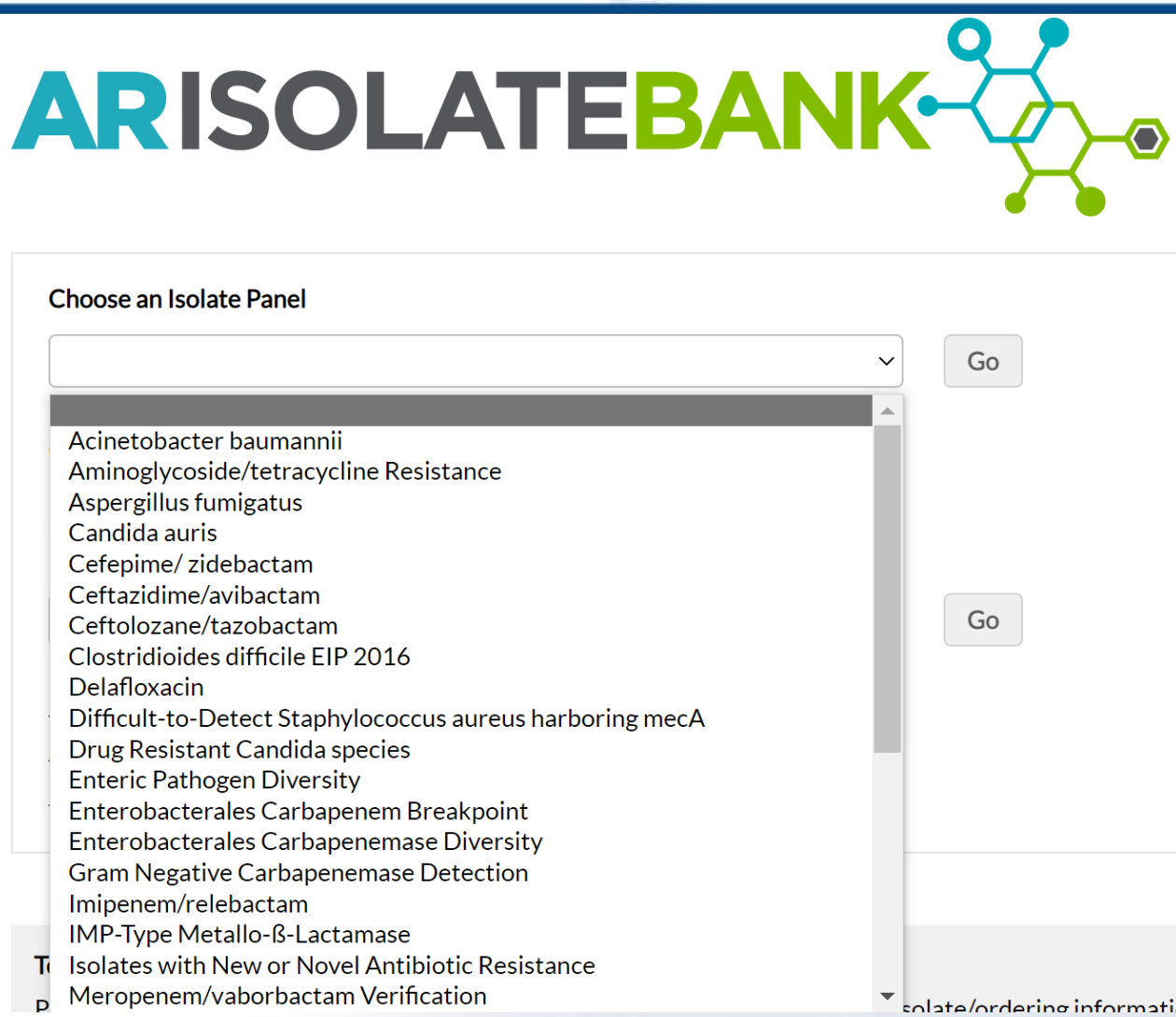
Method X	Diagnostic Accuracy Criteria		
	Positive	**Negative**	**Total**
Positive	# true positive TP	# false positive FP	TP + FP
Negative	# false negative FN	# true negative TN	FN + TN
Total	TP + FN	FP + TN	N

$$\text{Estimated Sensitivity (sens)} = 100 \times [TP / (TP + FN)] \quad (1)$$

$$\text{Estimated Specificity (spec)} = 100 \times [TN / (FP + TN)] \quad (2)$$

Validation/Verification Resources

- CDC FDA AR Bank Isolates
  - The CDC & FDA Antibiotic Resistance Isolate Bank | FDA
- Previously molecularly characterized isolates
- Reach out to the manufacturer they usually have verification panels available
- CLSI EP12-A2



Example Verification vs Validation

	Carba 5 LFA – On Label Use	Cepheid CARBA R –Off Label Use*
Use of Test	Test carbapenem-resistant Enterobacterales/ *P. aeruginosa* isolates	Test rectal Eswabs by CARBA R – 300 ul aliquot of Amies broth
Type of Study	Verification	Validation
Precision	Inter-repro: 5 positive and 1 negative over 3 days Intra-repro:5 positive and 1 negative 3 x in one day	Inter-repro: 5 positive and 1 negative over 3 days Intra-repro:5 positive and 1 negative 3 x in one day
Accuracy	30 isolates • 20 CP-CRO (VIM, IMP, OXA, NDM & KPC) • 10 non-CP-CRO	50 spiked-in isolates into rectal swab matrix • 30 CP-CRO (VIM, IMP, OXA, NDM & KPC) • 20 non-CP-CRO or off-target CP-CRO
Reportable range	Not applicable	Not applicable
Reference range	A negative result	A negative result
Analytical Sens	Not applicable	10^1 – 10^4 CFU/ml for 12 CP-CRO in triplicate
Analytical Spec	Not applicable	Addressed with accuracy (e.g., IMP-24 producer)

*Yee *et al*, EJCMID, 2021.

Now Let's Fast Forward to 2050

- What if we encounter Mrs. Anne Miller 2.0 with multidrug-resistant gram-negative bloodstream infection?
  - Will we have an antibiotic to treat her?
  - Will it be a story of success?
- We need to return our focus to tackling AMR globally, nationally and institutionally
  - We need to lobby to obtain the resources to tackle this important threat

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Summary

- AMR is a global public health concern that requires collective action
- Many different approaches can be applied to address AMR in the clinical microbiology laboratory
- Validation/verifications must be performed prior to implementing AMR tests for diagnostic purposes

Thank-you!

- Questions?
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  - Twitter @SimnerLab