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:
- 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
Let’s Rewind to March, 1942

- 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 War II for surgical and wound infections

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)
The Bugs are Always Smarter Than the Drugs

Antimicrobial Deployment

- Penicillin
- Tetracycline
- Chloramphenicol
- Streptomycin
- Erythromycin
- Vancomycin
- Ampicillin
- Methicillin
- Cephalosporins
- Linezolid
- Daptomycin

Antimicrobial Resistance Observed

- Penicillin
- Tetracycline
- Chloramphenicol
- Streptomycin
- Erythromycin
- Vancomycin
- Ampicillin
- Methicillin
- Cephalosporins
- Linezolid
- Daptomycin
The Bugs are Always Smarter Than the Drugs

Antimicrobial Deployment

First report of emergence of ceftazidime-avibactam resistance during treatment due to a mutation in the omega loop of the $\text{bla}_{\text{KPC-3}}$ gene (Shields, AAC, 2017; Shields, OFID, 2017)

First report of emergence of meropenem-vaborbactam resistance during treatment due to IS5 promoter insertion resulting in decreased $\text{ompK36}$ expression (Shields, CID, 2020)

Reports of emergence to cefiderocol resistance during treatment associated with mutations in the catecholate siderophore receptor $\text{cirA}$ (Klein, 2021, CID) or with increased copy number & expression of $\text{bla}_{\text{NDM-5}}$ (Simner, 2021, CID)
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.
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
## We Are Facing It in the Microbiology Laboratory

### Susceptibility

<table>
<thead>
<tr>
<th>Susceptibility</th>
<th>MIC</th>
<th>BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumoniae (K. pneumoniae)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin + Sulbactam</td>
<td>&gt;16/8 ug/mL</td>
<td>R</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>&gt;16 ug/mL</td>
<td>R</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>&gt;16 ug/mL</td>
<td>R</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>&gt;16 ug/mL</td>
<td>R</td>
</tr>
<tr>
<td>Cefepime</td>
<td>&gt;16 ug/mL</td>
<td>R</td>
</tr>
<tr>
<td>Cefotaxim</td>
<td>&gt;16 ug/mL</td>
<td>R</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;16 ug/mL</td>
<td>R</td>
</tr>
</tbody>
</table>

### Pseudomonas aeruginosa

<table>
<thead>
<tr>
<th>Susceptibility</th>
<th>MIC</th>
<th>BP</th>
<th>KB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa (P. aeruginosa)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>&gt;32 ug/mL</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>&gt;16 ug/mL</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Cefazolin</td>
<td>&gt;16 ug/mL</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Cefotaxim</td>
<td>&gt;16 ug/mL</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;2 ug/mL</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Clindamycin</td>
<td>&lt;1 ug/mL</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Colistin</td>
<td>&lt;1 ug/mL</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Daptomycin</td>
<td>&gt;8 ug/mL</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>&gt;8 ug/mL</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>&gt;8 ug/mL</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Lincomycin</td>
<td>&gt;8 ug/mL</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Meropenem</td>
<td>&gt;8 ug/mL</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Meropenem-Vaborbactam</td>
<td>&gt;16/8 ug/mL</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>32 ug/mL</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Piperacillin-Tazobactam</td>
<td>&gt;64/4 ug/mL</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>&gt;8 ug/mL</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Tobramycin</td>
<td>&gt;8 ug/mL</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Trimethoprim-Sulfamethoxazole</td>
<td>&gt;2/38 ug/mL</td>
<td>R</td>
<td></td>
</tr>
</tbody>
</table>
WHAT CAN WE DO TO TACKLE AMR IN LABORATORY MEDICINE?
Current Paradigm for ID & AST

Day 0
- Collection and plating of specimen in the lab

Day 1
- Isolation of your organism on solid media
  - MALDI-TOF MS ID
  - Set up of AST panels

Day 2
- Standard AST panel results available
  - Setup of additional antimicrobials

Day 3
- Additional AST results

**Average TAT: 2-3 days**

**Narrowed Treatment**

**Targeted Treatment**

AST: antimicrobial susceptibility testing; ID: identification; whole genome sequencing-AST: WGS to predict AST
Breakpoints = Stop Light Approach to Guide Therapy

- **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
Many New Toys in The Clinical Microbiology Laboratory

Proteomic Based ID: MALDI-TOF MS

Sophisticated Advanced NGS Technologies

CLIA waived PCR POC devices

Total Laboratory Automation

Rapid Phenotypic AMR or AST Methods

Moderately Complex Closed Systems- Sample- to-Answer devices

Syndromic Multiplex Molecular Panels
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
<table>
<thead>
<tr>
<th>Source</th>
<th>Test</th>
<th>AMR genes</th>
<th>TAT (hr)</th>
<th>FDA Status</th>
</tr>
</thead>
</table>
| Whole blood   | T2 Resistance                 | **meca/C, vanA/B, bla\textsubscript{CTX-M}, bla\textsubscript{KPC}, bla\textsubscript{NDM},**
|               |                               | **bla\textsubscript{VIM}, bla\textsubscript{IMP}, bla\textsubscript{OXA-23/OXA-48-like}, bla\textsubscript{CMY},**
|               |                               | **bla\textsubscript{DHA}**                                    | 3-5      |            |
| + Blood Cultures | Xpert MRSA/SA BC          | **meca**                                                      | 1        | ✓          |
|               | Biofire BC-IDII              | **meca, vanA/B, bla\textsubscript{CTX-M}, bla\textsubscript{KPC}, bla\textsubscript{NDM},**
|               |                               | **bla\textsubscript{VIM}, bla\textsubscript{IMP}, bla\textsubscript{OXA**} | 1        | ✓          |
|               | Verigene BC-GP & BC-GN       | **meca, vanA/B, bla\textsubscript{CTX-M}, bla\textsubscript{KPC}, bla\textsubscript{NDM},**
|               |                               | **bla\textsubscript{VIM}, bla\textsubscript{IMP}, bla\textsubscript{OXA**} | 2.5      | ✓          |
|               | Genmark BCID-GP & -GN        | **meca/C, vanA/B, bla\textsubscript{CTX-M}, bla\textsubscript{KPC}, bla\textsubscript{NDM},**
|               |                               | **bla\textsubscript{VIM}, bla\textsubscript{IMP}, bla\textsubscript{OXA-23/OXA-48-like}** | 1.5      | ✓          |
| Respiratory   | Biofire Panel Pneumonia      | **meca/C, MREJ vanA/B, bla\textsubscript{CTX-M}, bla\textsubscript{KPC},**
|               | Curetis Unyvero              | **bla\textsubscript{NDM}, bla\textsubscript{VIM}, bla\textsubscript{IMP}, bla\textsubscript{OXA-48},** expanded panel | 1        | ✓          |
|               |                               |                                                              | 4-5      | ✓          |
| Isolates      | Xpert Carba-R                | **bla\textsubscript{KPC}, bla\textsubscript{NDM}, bla\textsubscript{VIM}, bla\textsubscript{IMP}, bla\textsubscript{OXA-48-like},** expanded panel |          |            |
|               | OpGen Acuitas AMR panel WGS  | Comprehensive (known AMR)                                    |          |            |
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.
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

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)
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?
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

Day 0
Collection and plating of specimen in the lab

Day 1
Isolation of your organism on solid media
- MALDI-TOF MS ID
- Set up of AST panels

Day 2
Standard AST panel results available
- Setup of additional antimicrobials

Day 3
Additional AST results

Identification of S. aureus
- Same day MSSA vs MRSA call

Optimize Patient Treatment with Potential for Earlier Discharge

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)

Novel Agents Have Specific Niches

<table>
<thead>
<tr>
<th>Agent (*US FDA approved)</th>
<th>KPCs</th>
<th>NDMs</th>
<th>OXA-48-like</th>
<th>Carbapenem-resistant <em>P. aeruginosa</em></th>
<th>Carbapenem-resistant <em>A. baumannii</em></th>
<th><em>S. maltophilia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Aztreonam-avibactam</td>
<td></td>
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<tr>
<td>Cefiderocol*</td>
<td></td>
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</tr>
<tr>
<td>Ceftazidine-avibactam*</td>
<td></td>
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<tr>
<td>Cefotolozane-tazobactam*</td>
<td></td>
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<tr>
<td>Eravacycline*</td>
<td></td>
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<tr>
<td>Imipenem-relebactam*</td>
<td></td>
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<tr>
<td>Meropenem-vaborbactam*</td>
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<tr>
<td>Plazomicin*</td>
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</tbody>
</table>

Always confirm activity with antimicrobial susceptibility testing!
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

<table>
<thead>
<tr>
<th>Source of Infection</th>
<th>Preferred Treatment</th>
<th>Alternative Treatment if First-line Options Not Available or Tolerated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystitis</td>
<td>Ciprofloxacin, levofloxacin, trimethoprim-sulfamethoxazole, nitrofurantoin, or a single dose of an aminoglycoside</td>
<td>Cefazidime-avibactam, meropenem-vaborbactam, imipenem-clastatin-relebactam, and cefiderocol Colistin (when no alternative options are available)</td>
</tr>
<tr>
<td>Pyelonephritis or complicated urinary tract infection?</td>
<td>Cefazidime-avibactam, meropenem-vaborbactam, imipenem-clastatin-relebactam, and cefiderocol Meropenem (extended infusion): only if ertapenem-resistant, meropenem-susceptible, AND carbapenemase testing results are either not available or negative</td>
<td>Once-daily aminoglycosides</td>
</tr>
<tr>
<td>Infections outside of the urinary tract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant to ertapenem, susceptible to meropenem, AND carbapenemase testing results are either not available or negative</td>
<td>Meropenem (extended infusion)</td>
<td>Cefazidime-avibactam</td>
</tr>
<tr>
<td>Infections outside of the urinary tract</td>
<td>Cefazidime-avibactam, meropenem-vaborbactam, and imipenem-clastatin-relebactam</td>
<td>Cefiderocol</td>
</tr>
<tr>
<td>Resistant to ertapenem, resistant to meropenem, AND carbapenemase testing results are either not available or negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acinetobacter baumannii carbapenemase identified</td>
<td>Cefazidime-avibactam, meropenem-vaborbactam, imipenem-clastatin-relebactam</td>
<td>Cefiderocol</td>
</tr>
</tbody>
</table>
| Metallo-
| β-lactamase (ie, NDM, VIM, IMP) carbapenemase identified | Cefazidime-avibactam + aztreonam, cefiderocol                                        | Cefiderocol                                                                            |
| OXA-48-like carbapenemase identified                     | Cefazidime-avibactam                                                                | Cefiderocol                                                                            |


AMR | Recommended Treatment
---|---------------------
ESBL | Meropenem
AmpC | Cefepime
Carbapenemase | ≠ based on genotype
Methods to Detection CP-CRO

Phenotypic Detection Methods

A. Modified Hodge Test
- 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.

B. Carba NP and variants

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

Molecular Detection Methods

J. Lateral Flow Immunoassay
- CARBA
  - Control line
  - KPC
  - OXA-48 like
  - VIM
  - IMP
  - NDM

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

### 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

### Drug Susceptibility

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>CTX-M</th>
<th>IMP</th>
<th>KPC</th>
<th>Pan Candida</th>
<th>Pan Gram-Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meropenem</td>
<td>🟦</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftazidime/avibactam</td>
<td>🟦</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefepime</td>
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</tbody>
</table>

Consult ID/ASP
Our Algorithm at JHH for CRE

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

If mCIM positive – setup the CARBA 5 LFA
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
<table>
<thead>
<tr>
<th>Organism</th>
<th>Cascade Rule</th>
<th>Agents Released or Setup Upon Cascade</th>
<th>Additional Agents Available Upon Request</th>
</tr>
</thead>
</table>
| **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: Enterobacterales (not including *Salmonella/Shigella)*

<table>
<thead>
<tr>
<th>Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting</th>
<th>Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution</th>
<th>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</th>
<th>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</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>Cefuroxime</td>
<td>Cefiderocol</td>
<td></td>
</tr>
<tr>
<td>Cefazolin</td>
<td>Ceftriaxone</td>
<td>Ceftazidime-avibactam</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime* or Ceftriaxone*</td>
<td>Cefepime†</td>
<td>Imipenem-relebactam</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Meropenem-vaborbactam</td>
<td></td>
</tr>
<tr>
<td>Amoxicillin-clavulanate</td>
<td>Tobramycin</td>
<td>Aztreonam</td>
<td></td>
</tr>
<tr>
<td>Ampicillin-sulbactam</td>
<td>Amikacin</td>
<td>Ceftaroline*</td>
<td></td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td></td>
<td>Ceftazidime*</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>Plazomicin</td>
<td>Ceftolozane-tazobactam</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Levofoxacin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefotetan</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefoxitin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracycline†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine only</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefazolin (surrogate for uUTI)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fosfomycin* (Escherichia coli)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: MDROs, multi-drug resistant organisms; uUTI, uncomplicated urinary tract infection
HOW WOULD YOU VERIFY/VALIDATE A METHOD TO DETECT AMR?
## Validation/Verification Plan

<table>
<thead>
<tr>
<th></th>
<th>Validation</th>
<th>Verification</th>
<th>Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Performed to <strong>establish</strong> the performance characteristics</td>
<td>Performed to <strong>verify</strong> the performance characteristics</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Precision</strong></td>
<td>Inter- (3 samples over 3 days) &amp; Intra-assay (3 samples run 3 times on the same day) reproducibility</td>
<td>Inter- &amp; Intra-assay reproducibility</td>
<td>≥ 95% precision</td>
</tr>
<tr>
<td><strong>Accuracy</strong></td>
<td>≥20 positive, 10 negative</td>
<td>20 positive, 10 negative</td>
<td>≥ 90% percent positive agreement (i.e., sensitivity)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>≥ 90% percent negative agreement (i.e., specificity)</td>
</tr>
<tr>
<td><strong>Reportable range</strong></td>
<td>Not applicable (NA)</td>
<td>NA</td>
<td>Does not apply to qualitative assays</td>
</tr>
<tr>
<td><strong>Reference range</strong></td>
<td>Usually a negative result</td>
<td>Usually a negative result</td>
<td>Usually a negative result</td>
</tr>
<tr>
<td><strong>Analytical sensitivity</strong></td>
<td>Serial dilutions of samples with known concentrations in triplicate</td>
<td>NA</td>
<td>LOD is the lowest concentration where all 3 replicates are detected</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Detection of only the intended targets</td>
</tr>
<tr>
<td><strong>Analytical specificity</strong></td>
<td>A panel of potentially interfering or cross-reacting AMR genes</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>
# 2x2 Contingency Table & Accuracy Calculations

## Table 1. 2 x 2 Contingency Table

<table>
<thead>
<tr>
<th>Method Being Evaluated</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td># true positive TP</td>
<td># false positive FP</td>
<td>TP + FP</td>
</tr>
<tr>
<td>Negative</td>
<td># false negative FN</td>
<td># true negative TN</td>
<td>FN + TN</td>
</tr>
<tr>
<td>Total</td>
<td>TP + FN</td>
<td>FP + TN</td>
<td>N</td>
</tr>
</tbody>
</table>

Estimated Sensitivity (sens) = \[100 \times \left(\frac{TP}{TP + FN}\right)\]  
Estimated Specificity (spec) = \[100 \times \left(\frac{TN}{FP + TN}\right)\]
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
<table>
<thead>
<tr>
<th>Use of Test</th>
<th>Carba 5 LFA – On Label Use</th>
<th>Cepheid CARBA R – Off Label Use*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test of Use</td>
<td>Test carbapenem-resistant Enterobacterales/ P. aeruginosa isolates</td>
<td>Test rectal Eswabs by CARBA R – 300 ul aliquot of Amies broth</td>
</tr>
<tr>
<td>Type of Study</td>
<td>Verification</td>
<td>Validation</td>
</tr>
<tr>
<td>Precision</td>
<td>Inter-repro: 5 positive and 1 negative over 3 days Intra-repro: 5 positive and 1 negative 3 x in one day</td>
<td>Inter-repro: 5 positive and 1 negative over 3 days Intra-repro: 5 positive and 1 negative 3 x in one day</td>
</tr>
<tr>
<td>Accuracy</td>
<td>30 isolates • 20 CP-CRO (VIM, IMP, OXA, NDM &amp; KPC) • 10 non-CP-CRO</td>
<td>50 spiked-in isolates into rectal swab matrix • 30 CP-CRO (VIM, IMP, OXA, NDM &amp; KPC) • 20 non-CP-CRO or off-target CP-CRO</td>
</tr>
<tr>
<td>Reportable range</td>
<td>Not applicable</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Reference range</td>
<td>A negative result</td>
<td>A negative result</td>
</tr>
<tr>
<td>Analytical Sens</td>
<td>Not applicable</td>
<td>$10^1 - 10^4$ CFU/ml for 12 CP-CRO in triplicate</td>
</tr>
<tr>
<td>Analytical Spec</td>
<td>Not applicable</td>
<td>Addressed with accuracy (e.g., IMP-24 producer)</td>
</tr>
</tbody>
</table>

*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
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?
  – Feel free to e-mail me: psimner1@jhmi.edu
  – Twitter @SimnerLab