New Efforts in Combating Antibiotic Resistance – The Antibiotic Resistance Laboratory Network

Paula Snippes Vagnone MT(ASCP)

July 11, 2017

MN Laboratory System Webinar Series
Objectives

- Describe the ARLN, including Minnesota’s role and testing capacity as a regional lab
- Outline the goals of the ARLN
- Review laboratory methods for the detection of carbapenemases
- Describe bacterial and fungal resistance threats
Overview of CDC’s New Antibiotic Resistance Laboratory Network
Problem: Gaps in State, Regional & National Lab Capacity

- Limited state capacity to detect and respond to known or emerging forms of resistance
- CDC acts as one of the few sentinel surveillance programs for emerging resistance
  - Highly reliant on an informed laboratorian or clinician to flag unusual resistance
- When AR mechanism testing does happen, it is performed at CDC, some state public health labs or at academic/corporate entities that are not connected to public health action
- Increasing reliance on culture independent diagnostic testing in clinical labs will limit public health detection of AR
Problem: Gaps in State, Regional & National Lab Capacity

CARB Action Plan Outlines Milestones to Improve Laboratory Capacity

Sub-Objective 2.1.1: Create a regional public health laboratory network that uses standardized testing platforms to expand the availability of reference testing services, characterize emerging resistance patterns and bacterial strains obtained from outbreaks and other sources, and facilitate rapid data analysis and dissemination of information.
CDC Antibiotic Resistance Threat Report

HAZARD LEVEL URGENT

- *Clostridium difficile (C. difficile)*
- Carbapenem-resistant *Enterobacteriaceae* (CRE)
- *Drug-resistant Neisseria gonorrhoeae* (cephalosporin resistance)

HAZARD LEVEL SERIOUS

- Multidrug-resistant Acinetobacter
- Drug-resistant *Campylobacter*
- Fluoroquinolone-resistant *Candida* (a fungus)
- Extended-spectrum β-lactamase producing *Enterobacteriaceae* (ESBLs)
- Vancomycin-resistant *Enterococcus* (VRE)
- Multidrug-resistant *Pseudomonas aeruginosa*
- Drug-resistant *Non-typhoidal Salmonella*
- Drug-resistant *Salmonella Typhi*
- Drug-resistant *Shigella*
- *Methicillin-resistant Staphylococcus aureus* (MRSA)
- Drug-resistant *Streptococcus pneumoniae*
- Drug-resistant tuberculosis (MDR and XDR)

HAZARD LEVEL CONCERNING

- Vancomycin-resistant *Staphylococcus aureus* (VRSA)
- Erythromycin-resistant *Streptococcus Group A*
- *Clindamycin-resistant Streptococcus Group B*
Microorganisms with a threat level of URGENT:
Clostridium difficile, drug-resistant Neisseria gonorrhoeae, and CRE
Solution: CDC’s AR Laboratory Network (ARLN)

- Transform the national lab infrastructure with regional laboratories and local labs with gold-standard methods and technology
- Enhanced testing capacity in all 50 states and five local jurisdictions
- Faster detection for rapid and improved public health response
- Communication channels to engage clinical laboratory partners
- Real-time, actionable data to combat AR threats
AR Solutions at Every Level

The ARLN ensures more consistent and improved communication, coordination, and tracking at all levels every time.

- When resistance threats are detected within healthcare facilities or state/local labs, regional labs can provide support to characterize, support response, and track these discoveries.
- Flexibility in surveillance testing to focus on the next emerging threat.
- CDC’s ARLN team and Programs provide logistics support, subject matter expertise, and tailored solutions.
A Look at the Seven ARLN Regional Labs (FY16)

- **WEST**
  - Washington State Public Health Laboratories
  - Core Testing
  - Candida
  - *N. gonorrhoeae*

- **CENTRAL**
  - Minnesota Department of Health Public Health Laboratory
  - Core Testing
  - Candida
  - *C. difficile*
  - Reflex Culture pilot
  - *S. pneumoniae*

- **MOUNTAIN**
  - Texas Department of State Health Services Laboratory
  - Core Testing
  - *N. gonorrhoeae*

- **MIDWEST**
  - Wisconsin State Laboratory of Hygiene
  - Core Testing
  - Reflex Culture pilot
  - *S. pneumoniae*

- **NORTHEAST**
  - Wadsworth Center Bacteriology Laboratory
  - Core Testing
  - Candida

- **MID-ATLANTIC**
  - Maryland Public Health Laboratory
  - Core Testing
  - *N. gonorrhoeae*

- **SOUTHEAST**
  - Tennessee State Public Health Laboratory
  - Core Testing
  - Candida
  - *N. gonorrhoeae*
  - Reflex Culture pilot
ARLN Testing in 50 States and Five Major Cities

• Carbapenem-R *Enterobacteriaceae* (CRE)
  • CRE isolate characterization for species, carbapenemase production and resistance profile

• Carbapenem-R *Pseudomonas aeruginosa* (CRPA)
  • CRPA isolate characterization for carbapenemase and resistance profile
A RLN Testing in 50 States and Five Major Cities

Suspected CRE/CRPA isolates are forwarded to State PHLs

Testing at the State/Jurisdictional PHL may include:
• Species confirmation
• Antimicrobial susceptibility testing confirmation
• Phenotypic screening for carbapenemase production
• Molecular detection of mechanism

Isolates with suspected novel resistance*

*Positive for carbapenemase production by phenotypic methods and negative by PCR; Alert sent to state HAI coordinator and CDC within 1 day
1. CRE/CRPA Isolate Characterization

2. Targeted surveillance
   - Carbapenem-R *Acinetobacter* spp.
   - ESBL-producing *Enterobacteriaceae*
     - Isolate testing for *mcr*-mediated colistin resistance

3. Outbreak Response
   CRE Colonization Screening
   - Confirms CRE/CRPA
   - Submits to HAI Coordinator
   - Identifies Patient Contacts
   - Coordinates Swab Collection
   - CRE/CRPA Colonization Screening from Rectal Swabs
   - Results to Facility, Epidemiologist, and Lab in 2 Days
ARLN Regional Lab Supplemental Testing

**Candida spp. AST**

- *C. glabrata, C. haemulonii, C. auris*
- Currently: 4 labs
- Fall 2017: All labs, plus *C. auris* colonization

**Streptococcus pneumoniae**

**Neisseria gonorrhoeae**

**Clostridium difficile**
Lab Capacity Supported by the AR Solutions Initiative: Testing & Coordination by CDC

<table>
<thead>
<tr>
<th>Healthcare Labs</th>
<th>State/Local Labs</th>
<th>Regional Labs</th>
<th>CDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Develop testing methods and guidance</td>
<td>• Provide strategic prevention recommendations</td>
<td>• Identify data gaps</td>
<td></td>
</tr>
<tr>
<td>• Conduct hi-resolution sequencing and confirmatory testing of unusual isolates for regional labs</td>
<td>• Request threat assessments</td>
<td>• Add new panels to the AR Isolate Bank for drug and diagnostic test development</td>
<td></td>
</tr>
<tr>
<td>• Collect findings from state and regional labs</td>
<td>• Identify trends in resistance</td>
<td>• Provide technical assistance (training and proficiency)</td>
<td></td>
</tr>
<tr>
<td>• Report critical findings to international partners</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Identify trends in resistance</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FDA-CDC Antibiotic Resistance Isolate Bank
https://www.cdc.gov/drugresistance/resistance-bank/index.html
Impact: Nationwide Testing to Fill Data Gaps, Inform Prevention & Response

<table>
<thead>
<tr>
<th>Public Health Priority</th>
<th>Current Lab Capacity</th>
<th>With the ARLN</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gonorrhea Testing</strong></td>
<td>6,000 isolates per year by all state health departments</td>
<td>20,000 isolates per year by four regional labs</td>
</tr>
<tr>
<td><strong>CRE Characterization</strong></td>
<td>8 states conduct testing through the EIP</td>
<td>All 50 states conduct characterization testing, regional labs to confirm especially unusual resistance</td>
</tr>
<tr>
<td><strong>CRE Outbreak Lab Support</strong></td>
<td>Upon request Provided by CDC to states</td>
<td>Sustained capacity Provided by all regional labs and CDC</td>
</tr>
<tr>
<td><strong>Detecting new resistance threats, like mcr-1 and C. auris</strong></td>
<td>Reported ad hoc Often detected first in academia</td>
<td>Sentinel surveillance Sustained, adaptable capacity to identify and address new AR threats</td>
</tr>
</tbody>
</table>
ARLN in Action

• Strategy and coordination for increased and sustained capacity
• Enhanced epi/lab collaboration at all levels
• Clinical lab partners for isolate sharing
• Improved communication among partners
• Improved coordination at CDC
• Rapid response for AR prevention

EARLY SUCCESSES

• 861 suspected CRE Isolates: 289 Confirmed, 3 mcr-1 cases
• 6 CRE colonization point prevalence surveys
• National infrastructure for detecting C. auris
• 1500 gonorrhea isolates: 300 sequenced, resistance trend tracking
• Novel C. difficile data to interrupt transmission
• Enhancing national capacity for S. pneumoniae testing
ARLN Testing

- Goal is to complement testing performed in clinical labs
- Not meant to displace or discourage extended testing in hospitals
- ARLN testing is a source of data for local and regional prevention efforts
- Laboratories and facilities will receive data and results for isolates submitted to the ARLN for testing
Detecting OLD Resistance Threats:
CRE & CRPA
The Enterobacteriaceae

- Facultatively anaerobic, Gram-negative bacilli
- Enteric organisms
- Common human pathogens
  - Urinary tract infections
  - Bacteremia
  - Pneumonia
  - Wound infections
- *Klebsiella, Escherichia coli, Enterobacter, Serratia, Citrobacter*
Carbapenem Antibiotics

• Newest class of FDA-cleared beta-lactamase antibiotics
• Broad spectrum activity
• Usually reserved as “antibiotics of last resort”
• Used to treat hospitalized patients with multi-drug-resistant bacterial infections
• Bacterial resistance to carbapenems is increasing
Emerging problem worldwide

Resistance generally plasmid mediated, highly transferrable through mobile genetic elements

Spread facilitated by ease of travel and medical treatment in endemic areas

Lack of proper patient screening or communication between facilities also increases spread

Long-term carrier state
Importance of CRE

- Infections are difficult to treat
- Emergence of pan-resistant strains
- New antibiotics are slow to develop
- Invasive infections are associated with high mortality rates
- Infections have risen sharply among patients in healthcare facilities
- Resistance can spread to other bacteria (CP-CRE)
**Terminology**

**CRE**: Carbapenem-Resistant Enterobacteriaceae
- Example: MIC values from ATI or manual method

**CP-CRE**: Carbapenemase-Producing, Carbapenem-Resistant Enterobacteriaceae
- Example: Test for carbapenemase production (e.g. CIM, MHT, Carba NP)

**KPC**: *Klebsiella pneumoniae* carbapenemase
- Example: Test for presence of specific carbapenemase-producing gene (e.g. PCR)
• Many mechanisms of carbapenem-resistance exist; some isolates encode multiple mechanisms
  • Plasmid-mediated carbapenemase genes
    • KPC, NDM, OXA-48, IMP, VIM, etc.
  • AmpC (intrinsic/plasmid-mediated + porin loss mimics)
    • Also, ESBLs

• Can be difficult to determine from AST profile alone
Carbapenem-Resistant Enterobacteriaceae (CRE)

- CARBAPENEMASE-PRODUCING CRE
  - KPC*
  - NDM*
  - OXA
  - VIM
  - IMP

- NON-CARBAPENEMASE-PRODUCING CRE
  - ESBL (+ porin loss)
  - AmpC (+ porin loss)
  - Other mechanisms

*Routinely tested for at MDH PHL
The Big 5 Carbapenemases

1. **Klebsiella pneumoniae carbapenemases (KPC)**
   - Most common carbapenemases in the United States
   - Confers resistance to ALL β-lactam agents

2. **New Delhi Metallo-β-lactamase (NDM)**
   - 1\textsuperscript{st} detected in 2009
   - Rapid global spread, primarily among Enterobacteriaceae
   - Previously associated with travel, now with domestic transmission
3. **OXA-48-like enzymes**
   - Inefficient, do not hydrolyze cephalosporins well
   - Commonly travel with other β-lactamases

4. **Verona Integron-encoded Metallo-β-lactamase (VIM)**
   - Relatively slow global spread
   - Mostly in *P. aeruginosa*

5. **Active on Imipenem Metallo-β-lactamase (IMP)**
   - Relatively slow global spread
   - Mostly in *P. aeruginosa* – *Providencia rettgeri*??
Patients with KPC-producing CRE reported to CDC as of January 1, 2017

https://www.cdc.gov/hai/organisms/cre/trackingcre.html
What do we see in MN?
Minnesota CRE Surveillance

• 2009: first KPC-producing CRE identified in MN
• Initiated passive statewide CRE surveillance with voluntary isolate submission
• In 2011 MDH initiated active CRE surveillance in Hennepin and Ramsey Counties
PCR Results for Enterobacteriaceae Isolates with Reduced Susceptibility to Carbapenem Antibiotics, 2009-2016

Species: *Escherichia coli*, *Enterobacter* spp., and *Klebsiella* spp.

Initiated active surveillance Statewide January 2016

Initiated active surveillance in Hennepin and Ramsey Counties, June 2011

Species: *Escherichia coli*, *Enterobacter* spp., and *Klebsiella* spp.
Carbapenemase producers 2016

- KPC: 74%
- NDM: 20%
- VIM: 4%
- IMP: 2%

n=53
CP-CRE and Non-CP-CRE Case Isolates by Species, Minnesota, 2014

Number of isolates (n=141)

CP-CRE
Non-CP-CRE
Not Tested

C. freundii 13
E. aerogenes 1
E. cloacae 10
E. coli 1
K. oxytoca 2
K. pneumoniae 6
Other 7

Other
CP-CRE Case Isolates by Specimen Type Minnesota 2014

- Urine: 8
- Blood: 3
- Respiratory: 1
- Wound: 1
- Other sterile site: 1
- Other non-sterile site: 1

n = 21
CRE Reportable in Minnesota
January 1, 2016
# MN CRE Reporting/Submitting Criteria

<table>
<thead>
<tr>
<th>Past Criteria</th>
<th>New Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species</strong></td>
<td><em>Klebsiella</em> spp., <em>Enterobacter</em> spp., <em>E. coli</em> and <em>Citrobacter</em> spp.</td>
</tr>
<tr>
<td><strong>Culture sites</strong></td>
<td>All body sites (sterile/non-sterile)</td>
</tr>
<tr>
<td><strong>Definition</strong></td>
<td>All body sites (sterile/non-sterile)</td>
</tr>
<tr>
<td>• Nonsusceptible to a carbapenem antibiotic*</td>
<td>• Resistant to any carbapenem antibiotic*: imipenem, meropenem, doripenem, or ertapenem</td>
</tr>
<tr>
<td>• Resistant to 3rd generation cephalosporins</td>
<td>• Demonstrates production of a carbapenemase (i.e. CIM, MHT)</td>
</tr>
</tbody>
</table>

*According to current CLSI guidelines*
Resistance is based on current Clinical Laboratory Standards Institute (M100) guidelines

- Challenge: many labs have not adopted the current carbapenem breakpoints
- May have to visually review results (MICs) to assess resistance
- Special queries or flags may be useful in LIMS or Automated Systems
### "New" CLSI Carbapenem Susceptibility Interpretive Criteria for Enterobacteriaceae

<table>
<thead>
<tr>
<th>Agent</th>
<th>CLSI M100-S19: 2009</th>
<th>CLSI M100-S24: 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>≤2</td>
<td>4</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤4</td>
<td>8</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≤4</td>
<td>8</td>
</tr>
<tr>
<td>Doripenem</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Laboratory Methods for Carbapenemase Detection
Clinical Lab Detection of Carbapenemases

• Current methods:
  • CIM Test
  • Carba NP test (not FDA cleared)
  • Modified Hodge Test
  • Etest strips with inhibitors
  • CHROM Agars (not FDA cleared)
Reference or Public Health Laboratory Detection of Carbapenemases

- CIM Test
- KPC/NDM multiplex PCR
- PCR for additional mechanisms
  - Check Points system (Not FDA cleared)
- Whole genome sequencing
CIM – Carbapenem Inactivation Method

Suspend full loop of bacteria in H₂O
Add 10 μg meropenem disk
Incubate for 2 hours 35°C
Place on Mueller Hinton agar inoculated with E. coli ATCC 25922

+ Carbapenemase activity
- No carbapenemase activity

mCIM
- TSB
- Smaller loopful
- 4 hours

Incubate for at least 6 hours 35°C
Read presence or absence of inhibition zone
CIM – Carbapenem Inactivation Method
The Carba NP test

- Rapid colorimetric biochemical screening test to detect carbapenemase production

- Each tube contains: Lysis buffer with bacterial suspension, phenol red pH indicator, zinc solution, ± antibiotic (imipenem)

1 Set of Reaction Tubes Pre-inoculation

[Images of reaction tubes with positive and negative results after 37°C, 2 hrs]

Carbapenemase Positive Isolate

Carbapenemase Negative Isolate
The Modified Hodge Test: Examples

• Limitations with MBL detection

• Can observe false positives with AmpC producers
  (Enterobacter spp.)
The Modified Hodge Test: Examples

Negative
Carbapenemase Real-Time PCR Assays

• Clinical labs directly detecting targets in blood cultures
  • Nanosphere Verigene®
  • Biofire®

• Targeted PCR
  • Carbapenemases: KPC, NDM (validated at MDH)
  • Carbapenemases*: VIM, IMP, OXA-48
  • ESBLs*: CTX-M, SHV, TEM

• Cepheid X-pert CarbaR
  • KPC, NDM, PZA-48-like, VIM, IMP-1

*Available at MDH, not validated
The MDH-PHL requests the following for each isolate submission:

1. CRE Isolate: a pure, low passage culture (RT or refrigerated)
2. Clinical Testing and Submission form
3. MDH CRE Isolate Submission Form
4. AST Report Printout
How and What to Submit - CRE
Clinical Testing and Submission Form

Be sure to include:

- project number
  - 2175 - MN
  - 1380 - Metro
- patient information
- specimen source
- collection date
- isolate genus/species

http://www.health.state.mn.us/divs/phl/clin/print_mdh.pdf
How and What to Submit - CRE
MDH CRE Isolate Submission Form

Be sure to include any AVAILABLE CRE test results:

- Commercial AST System
- Modified Hodge Test results
- E-test Results
- Disk Diffusion Results
- Select antimicrobial agent results
- Results from other tests performed (i.e. Carba NP, PCR)
How and What to Submit - CRE
AST Report Printout

Be sure to submit MIC profile obtained from your AST Instrument

i.e. Vitek2, Microscan, Phoenix

Note: Make sure to submit the raw data from your testing platform; not a printout from a patient chart

<table>
<thead>
<tr>
<th>Name</th>
<th>MIC</th>
<th>Interps</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacter cloacae</td>
<td>&lt;4</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>&gt;16</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Aztreonam</td>
<td>&gt;16</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Cefazolin</td>
<td>&gt;16</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Cefepime</td>
<td>&gt;16</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>&gt;32</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime/K C...</td>
<td>&gt;4</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Cefotetan</td>
<td>&gt;32</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>&gt;16</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>&gt;16</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Ceftazidime/K ...</td>
<td>&gt;2</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>&gt;32</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>&gt;16</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Cephalothin</td>
<td>&gt;16</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>16</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;2</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>8</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>&gt;8</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>&gt;4</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Meropenem</td>
<td>&gt;8</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>&gt;64</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Pip/Tazo</td>
<td>&gt;64</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Piperacillin</td>
<td>&gt;64</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>&lt;4</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Ticar/K Clav</td>
<td>&gt;64</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Tobramycin</td>
<td>&gt;8</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Trimeth/Sulfa</td>
<td>&gt;2/38</td>
<td>R</td>
<td></td>
</tr>
</tbody>
</table>
Suspect CRE
Isolate Submitted

Examination of AST Report
(Microscan, etc.) from submitter – **Meets Criteria**

**YES**
Perform CIM Test

**POSITIVE**
KPC/NDM real-time PCR performed

**NEGATIVE**
Carbapenemase Negative

---

**NO**
Isolate identification confirmed (MALDI-TOF, biochemical)

**POSITIVE**
Isolate ID and KPC/NDM results reported to submitter

**NEGATIVE**
Additional carbapenemase testing performed
What Should Clinical Labs be Doing?

- At minimum, compare isolate MICs with 2016 CRE definition
  - Use updated CLSI guidelines for interpretations
  - Consult expert comments from your system (if available)

<table>
<thead>
<tr>
<th></th>
<th>CLSI M100-S24 (2014)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agent</td>
<td>S</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>≤0.5</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤1</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≤1</td>
</tr>
<tr>
<td>Doripenem</td>
<td>≤1</td>
</tr>
</tbody>
</table>

- Perform in-house phenotypic testing (i.e. CIM, if available)
- Molecular testing (if available)
- Submit suspicious isolates to MDH-PHL
- Call MDH for consultation 651-201-5581
- Rectal swab cultures for patients receiving healthcare abroad
Detecting New Resistance Threats: *Candida auris*
Fluconazole-resistant *Candida* included as “serious threat” in the 2013 CDC AR threat report.

Fluconazole is the most readily available and frequently prescribed antifungal.

Resistance varies by species:
- *C. glabrata* (high), *C. albicans* (low)

• Increase capacity to identify and track antifungal resistance among *Candida* species and detect new and emerging resistant species, such as *Candida auris*. 
Actively looking for echinocandin resistance

*C. glabrata* has both azole resistance and about 2-6% echinocandin resistance with some hospitals recording up to 30%

Echinocandins have only been in use for <20 years and we do not yet know how resistance will trend

Nearly 0% resistance for *C. albicans, C. dubliniensis, C. tropicalis, C. parapsilosis, C. lusitaniae, C. krusei* so we are not collecting those species
• Goal to include a variety of health care facilities (large, small, rural, urban)

• *Candida* sp. from **sterile body sites** (blood, CSF)

• **Submit**: *C. auris*, *C. haemulonii*, *C. glabrata* and other *Candida* species

• **Do Not Submit**: *C. albicans*, *C. dublinensis*, *C. krusei*, *C. parapsilosis*, *C. lusitaniae*, *C. tropicalis*

• Serial *C. glabrata* isolates requested to be collected to test for resistance development

• Isolates stable on slants and can be batched for shipment
Why is ARLN concerned about *C. auris*?

- Multi-drug resistant
  - Some isolates resistant to all 3 major antifungal classes
- Often misidentified
  - Usually misidentified as *C. haemulonii* or other *Candida* species
- Causes outbreaks in health care settings
  - Unlike other *Candida* spp., seems to colonize health care environments and skin
  - Results in major infection control challenges
ARLN *Candida* sp. Surveillance – *Candida auris*

**Clinical Alert to U.S. Healthcare Facilities - June 2016**

Global Emergence of Invasive Infections Caused by the Multidrug-Resistant Yeast *Candida auris*

*Summary:* The Centers for Disease Control and Prevention (CDC) has received reports from international healthcare facilities that *Candida auris*, an emerging pathogen, has caused invasive infections. *C. auris* is associated with high mortality. Some strains of *C. auris* have elevated resistance to many antifungal drugs, limiting treatment options. *C. auris* requires specialized identification methods. CDC is aware of one isolate of *C. auris* in the United States, and multiple isolates in other countries.

**Clinical Infectious Diseases**

Simultaneous Emergence of Multidrug-Resistant *Candida auris* on 3 Continents Confirmed by Whole-Genome Sequencing and Epidemiological Analyses

Clinical *C. auris* Cases by Date
May 2013 – May 2017
(n=86)

Courtesy Shawn Lockhart, PhD D(ABMM) Mycotic Disease Branch, CDC
ARLN *Candida* sp. Surveillance – *Candida auris*

*Candida auris* cases in the United States

Number of Cases Reported

- **1 - <16**
- **16 - <31**
- **31 - <45**
- **45 - 60**

https://www.cdc.gov/fungal/diseases/candidiasis/candida-auris.html
ARLN Candida sp. Surveillance – Candida auris Challenges

- Colonizes skin and other body sites
- Found on multiple surfaces in rooms when studied by CDC
- Close contacts get colonized with C. auris in a health care setting
- Quarternary ammonium compounds inadequate for disinfection
- Persists for >4 weeks on plastic surfaces when studied by CDC
**ARLN Candida sp. Surveillance – Candida auris Challenges**

- Commercial tests: API, BD Phoenix, MicroScan, Vitek-2 *C. auris* not in the libraries
- Most common mis-identifications:

<table>
<thead>
<tr>
<th>Identification Method</th>
<th>Common C. auris Misidentification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitek 2 YST</td>
<td><em>Candida haemulonii</em></td>
</tr>
<tr>
<td>BD Phoenix yeast identification system</td>
<td><em>Candida haemulonii</em> <em>Candida catenulata</em></td>
</tr>
<tr>
<td>Microscan</td>
<td><em>Candida catenulata</em> <em>Candida famata</em> <em>Candida guilliermondii</em> <em>Candida lusitaniae</em></td>
</tr>
<tr>
<td>API 20C</td>
<td><em>Rhodotorula glutinis</em> (and characteristic red color not present) <em>Candida sake</em></td>
</tr>
</tbody>
</table>
Can be identified correctly by:
  - DNA sequencing
  - MALDI-TOF, if added to the database

Likes high salinity and temperatures (>40°C)
For routine ARLN yeast surveillance:

- **Submit:** *C. auris*, *C. haemulonii*, *C. glabrata* and other *Candida* species
- **Do Not Submit:** *C. albicans*, *C. dublinensis*, *C. krusei*, *C. parapsilosis*, *C. lusitaniae*, *C. tropicalis*

For *C. auris* surveillance, notify MDH and submit:

- Isolates of *Candida* sp. from any body site per previous table
- *Candida* spp.
ARLN *Candida* sp. Surveillance – ARLN Regional Lab Testing

- Antifungal testing – TREK custom MBD panel, and Etest (Ampho-B)
- Yeast ID:
  - MALDI
  - DNA sequencing – CDC
• Antifungal testing – aggregate reports
• Yeast ID results will be received within 1 day of testing
Detecting New Resistance Threats: $mcr-1$
mcr-1 Colistin Resistance – ARLN Goal

• Collect isolates to allow for ongoing evaluation of this newly recognized or emerging mechanism of resistance

• Target labs serving agricultural areas
Why is ARLN concerned about *mcr*-1?

- Mediates resistance to colistin
- Colistin is in one of the last classes of antibiotics NOT having plasmid-mediated spread
mcr-1 Colistin Resistance - Background

• Scientists- routine surveillance project – AR in *E. coli* from food animals in China
• Noticed a major increase in colistin resistance
• Colistin widely used in China in animals for growth promotion
mcr-1 Colistin Resistance - Background

- Found plasmid-mediated spread of mcr-1 gene and it was causing resistance to colistin
- 166/804 (21%) animals
- 16/1322 (1%) human inpatient with infections
Likely that \textit{mcr}-1 originated in animals and subsequently spread to humans.

Predicted that \textit{mcr}-1 likely to spread rapidly into key human pathogens.

Colistin is not known to be used in animals in the U.S.
Since initial report, found globally

- > 20 countries and 6 continents
- Food animals, meat, vegetables, surface water
- Ill patients, asymptotically colonized patients

Multiple species: *E. coli*, *K. pneumoniae*, *Salmonella enterica*, *Shigella sonnei*
**mcr-1 Colistin Resistance – Emergence U.S.**

- **1st Report** – Pennsylvania (May, 2016)
  - ESBL *E. coli* from urine, outpatient
  - Travelled to Mexico 10 months prior
  - No animal contact

- **12 reports as of February 10, 2017**
  - 10 human isolates (*8 E. coli* and 2 *Salmonella*)
  - 2 porcine isolates collected at slaughter (*E. coli*)
mcr-1 Colistin Resistance – Emergence U.S.

Tracking mcr-1

https://www.cdc.gov/drugresistance/tracking-mcr1.html
• ~11% of ESBLs tested at CDC have colistin MIC ≥4 µg/ml
• *E. coli* (primarily) and *K. pneumoniae*
• Issues with colistin testing
• Only one isolate so far found to be CP-CRE
## mcr-1 Colistin Resistance – Susceptibilities, Isolates Prior to 12/31/17

<table>
<thead>
<tr>
<th></th>
<th>ESBL</th>
<th>Carbenamase</th>
<th>Colistin MIC</th>
<th>Ceftriaxone</th>
<th>Ceftazidime</th>
<th>Cefepine</th>
<th>Imipenem</th>
<th>Ertapenem</th>
<th>Doripenem</th>
<th>Meropenem</th>
<th>Tmp-Smx</th>
<th>Ciprofloxacin</th>
<th>Levofoxacin</th>
<th>Gentamicin</th>
<th>Tobramycin</th>
<th>Amikacin</th>
<th>Aztreonam</th>
<th>Piptazo</th>
<th>Ampicillin</th>
<th>Tigecycline</th>
<th>Amp-sulbactam</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>Y</td>
<td>Y</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>Y</td>
<td>N</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>Y</td>
<td>N</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>Y</td>
<td>N</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>Y</td>
<td>N</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>Y*</td>
<td>N</td>
<td>2^</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>N</td>
<td>N</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella Enteriditis</td>
<td>N</td>
<td>N</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella Typhirium</td>
<td>NT</td>
<td>NT</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- E-test for Colitin; MicroScan for all others
- AmpC
- Polymyxin B MIC = 4

**Susceptible**

**Intermediate**

**Resistant**

**Not tested**
mcr-1 Colistin Resistance – ARLN Isolate Submission Criteria

- *E. coli* and *Klebsiella* spp.
- Susceptible to all carbapenems
- Resistant to 3rd generation cephalosporins
  - Cefotaxime, ceftriaxone – MIC ≥ 4 µg/mL
  - Ceftazidime – MIC ≥ 16 µg/mL
- Positive test result for ESBL production, if performed at submitting lab
- AST – TREK Sensititre panel (GNX2F)
  - Elevated colistin MIC perform PCR for mcr-1/mcr-2
- Confirm ESBLs by disk diffusion – ceftazidime and cefotaxime with and without clavulanate
- CDC suggests testing isolates in batches at least twice per month
• Submitting labs will receive faxed reports
  • \textit{mcr-1/mcr-2} PCR
• TREK AST and ESBL phenotypic confirmation – \textbf{Will not be reported}
• Positive results will be received within 1 day of testing
Summary: CDC’s AR Laboratory Network (ARLN)

- **Transform the national lab infrastructure** with regional laboratories and local labs with gold-standard methods and technology
- **Enhanced testing capacity** in all 50 states and five local jurisdictions
- **Faster detection** for rapid and improved public health response
- **Communication channels** to engage clinical laboratory partners
- **Real-time, actionable data** to combat AR threats
Upcoming Audio Conferences

• Culture Independent Diagnostic Testing (CIDT) – August 15\textsuperscript{th}, 12-1pm

• Influenza Update – An overview of the upcoming influenza season – October, Exact date and time TBD

• Any other topic suggestions? Email us!
If you would like P.A.C.E credit for today’s audio conference, please contact Eric Lundquist: eric.Lundquist@state.mn.us.

You will be asked to fill out a brief evaluation.
Thank You!

MDH Public Health Laboratory
Microbiology Unit Supervisor
Paula Snippes Vagnone
651-201-5581
paula.snippes@state.mn.us