Indiana State Department of Health Laboratory guidance on implementing training

To promote enhanced detection of CP-CREs in the state of Indiana, the Indiana State Department of Health Laboratories (ISDHL) developed a series of workshops to teach the theory and practice of how to detect CP-CRE within the clinical laboratory setting. These trainings provide instruction on phenotypic and molecular methods of detection, as well as methods to assess patient colonization. By combining didactic theory with hands-on practical experience, attendees walk away with a complete training experience.

The ISDHL journey started in 2013 with a CRE Pilot Study. This pilot study was instrumental for guiding ISDHL program development and provided the data necessary to make CP-CRE reportable in Indiana. Specifically, the pilot project demonstrated that CP-CRE reporting rules should be CLSI-independent. Instead, relying on MIC- or zone diameter, a phenotypic screening recommendation, and guidelines should be written to allow for the emergence of newer technologies, such as molecular assays.

On December 25, 2015, the ISDH made CP-CRE reportable for condition, laboratory reporting, and isolate submission in Indiana. In order to facilitate awareness and enhance laboratory practices, the ISDHL developed and hosted a series of CP-CRE workshops starting in 2015. The CP-CRE workshop utilizes a pre- and post-test format to determine if knowledge gaps were addressed through the workshop materials. The workshops had three main goals: (1) understand the differences between CRE and CP-CRE and how this impacts transmission, (2) understand how to screen for CP-CRE and submit isolates to ISDHL for confirmation, and (3) teach laboratory methods for CP-CRE isolate confirmation and colonization screening.

As of May 2017, 46 microbiologists from 36 facilities have attended this workshop. When comparing pre- and post-workshop testing scores, participants scored an average of 47% higher on the post-test, indicating an increased understanding of the testing material after completing the workshop. Attending laboratories have also demonstrated a 19% increase in screening accuracy when compared with laboratories who have not yet attended, indicating workshop efficacy.

Vision:

This workshop is not just about the isolates; it's about engaging laboratorians on this emerging and evolving topic. If we've done our job, at the end of the workshop we not only have taught the students the techniques, but we've also engaged them in the problem. In general, this workshop aims to increase the level of awareness and communication between Indiana clinical laboratories and ISDHL on the topic of CP-CRE and antibiotic resistance.

Goals:

1. **Understand the differences between CRE and CP-CRE and how this impacts transmission**

   Purpose: CP-CRE are a global threat to public health due to the mobile nature of these enzymes, which are typically encoded upon plasmids. Carbapenemases confer resistance to all β-lactam antibiotics, including the carbapenems, which are often considered the last

line antibiotics for Gram negative infections. Carbapenemases are mobile resistance mechanisms, and thus present an increasing threat for infection control. Understanding the differences between CRE and CP-CRE (e.g. type of resistance mechanism) is important for proper detection, containment, and prevention of these multidrug-resistant organisms. In essence, the workshop aims to answer the question: what are CP-CREs, how do they differ from CREs, and what is the impact of this from the patient and public-health level?

Resources:

- Appendix A: Selected slides on Carbapenemase Producing – Carbapenem Resistant *Enterobacteriaceae*

2. Understand how to screen for CP-CRE and submit isolates to ISDHL for confirmation

Purpose: CP-CRE is isolate reportable in Indiana. In order to meet this requirement, laboratorians need to understand how to screen for CP-CRE, what qualifies as a CP-CRE, how quickly these isolates must be submitted, and how to submit these isolates to the state public health laboratory.

One of the main components of the Indiana Communicable Disease Reporting Rule is the inclusion of a phenotypic assessment of carbapenemase production, however, ISDHL's CRE pilot identified that many laboratorians were unsure of how to perform these tests required to identify carbapenemase production. Therefore, the training aims to increase the attendee's knowledge-base on use of these methods.

For Indiana: Laboratories must submit organisms that are

1.) Nonsusceptible to at least one (1) carbapenem antibiotic with an MIC ≥ 2 µg/mL or <=22 mm (<=21 mm for ertapenem) AND are positive for carbapenemase production by a phenotypic method OR

2.) Nonsusceptible to at least three (3) carbapenem antibiotics (with MIC >=2 µg/ml or zone diameter <=22 mm (<=21 mm for ertapenem) OR

3.) Positive for a carbapenemase gene maker.

These results are to be reported within 72 hours to the state health department AND the isolate must be submitted to the state laboratory for CRE characterization. Patients that are repeatedly positive with the same organism are not required to submit duplicate isolates.

Resources

- Appendix B: How to submit isolates to state laboratory
- Appendix C: ISDH Laboratories Reporting Requirements

3. **Learn some new (or old) lab methods**

   Purpose: Demonstrate both new and old techniques that clinical laboratories could implement to detect carbapenemases in their laboratory.

   The workshop explains the differences in testing methods for carbapenemase production. Detailed technical information on testing including the theory behind the testing methods is provided. Practical information, such as commercial availability of products, technician-time, cost-per-test, and training considerations are also provided. Limitations to the test method are discussed so that the laboratorians can be aware of potential scenarios that could cause false-positive and false-negative results and how to troubleshoot these scenarios when they occur. A hands-on bench training is then provided, allowing workshop participants to visualize the methods described in the didactic portion of the workshop.

   The workshop is structured to allow for time to discuss the many barriers to test implementation, including: how to perform validations/verifications of non-FDA-approved tests and determining the cost/benefit to implementing colonization screening. Big picture concepts are also discussed, such as: how to engage the laboratory administration, how to demonstrate the savings that the laboratory can provide by decreasing the number of patient days in isolation, and the role the laboratory plays in antibiotic stewardship.

   Resources

   - Appendix D: Laboratory Methods of Testing
   - Modified Hodge Test (MHT) for Carbapenemase Detection

Appendix A: Selected slides on CP-CRE

Carbapenemase Producting –
Carbapenem Resistant *Enterobacteriaceae*

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NATIONAL ACTION PLAN FOR COMBATING ANTIBIOTIC-RESISTANT BACTERIA

Epidemiology and Laboratory Capacity Cooperative Agreement

Antibiotic Resistance Threats in the United States, 2013

Klebsiella pneumoniae from a tracheal aspirate
Ertapenem R, imipenem R, meropenem R

Combination of AmpC, ESBL, porin mutations	KPC	NDM, VIM, IMP
Non-carbapenemase	Carbapenemase (serine-based)	Carbapenemase (Zinc-catalyzed)

What about all of these acronyms?

Organism Group	Glucose Non-Fermenters		Enterobacteriaceae	
Resistance Mechanism	Inherent	Carbapenemase	Hyper. of AmpC plus porin loss	Carbapenemase
CRE	–	–	Y	Y
CP-CRE				Y

CRE – Carbapenem Resistant *Enterobacteriaceae*
CP-CRE – Carbapenemase Producing-Carbapenem Resistant *Enterobacteriaceae*

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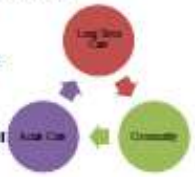
Appendix B: How to submit an isolate sample to the state laboratory

CP-CRE (isolate)	
Short Name	Carbapenemase Producing - Carbapenem Resistant Enterobacteriaceae
Specimen Requirements	1. Specimen type: Pure viable culture on appropriate agar medium slant. 2. One isolate per patient. 3. ISDH Communicable Disease Rule 410-IAC 1-2.3-76 requires submission of these isolates within (3) business days of isolation. 4. Temperature requirement: Ambient conditions.
Sampling Materials	1. Sample Container: Appropriate agar medium slant in tube with screw-cap tightened or other similarly approved commercial transport medium. 2. Shipping boxes/containers with appropriate shipping labels, commercially available.
Procedural Notes	1. Be sure to properly label each specimen tube with the patient's name, date of birth, and date of isolation. A minimum of two unique patient identifiers are required to be present on submitted specimen. 2. Check the expiration date on the tube to ensure the product is acceptable and will continue to be acceptable once received at the ISDH laboratory. 3. Complete the LimsNet submission form for CRE testing. LimsNet is available on the web at http://limsnet.isdh.in.gov. Users should call the lab's LIMS Help Desk to get access to this system. The Help Desk can be reached at 888-535-0011, or locally at 317-921-5506. Submitters can also email the Help Desk at LimsAppsuport@isdh.in.gov.
Shipping Instructions	Ship To: Indiana State Department of Health Laboratories 550 West 16th Street Indianapolis, IN 46202 1. Package according to Category B UN3373 triple contained in accordance with federal shipping regulations for infectious substances/diagnostic specimens. 2. Tighten the specimen container tube caps. 3. Label clearly on each specimen tube with the patient name, date of birth, and date of isolation. 4. Wrap each labeled, primary/specimen container tube with absorbent material. Place each primary container tube with absorbent material into the inner mailing container and tighten the cap securely. 5. The completed submission/request form may then be wrapped around the sealed inner container and together placed securely into the outer shipping container. 6. Clearly label the outer container with the senders name/address and recipients name/address. 7. Do not send culture isolates on petri plates if submitting by mail. 8. Transport Temperature: Ambient conditions.
Reporting and TAT	1. Reporting Method: LimsNet 2. TAT (ISDH Testing): 2 business days. 3. Test Referral. Cultures identified as possible carbapenemase producing isolates with unusual profiles will be sent to the CDC for confirmation and/or further testing. Projected TAT listed above does not account for time required for isolate submission to the CDC.
Lab	Clinical Microbiology
Keywords	CP-CRE, Carbapenemase Producing - CRE
Fee, if applicable	Not applicable.

Appendix C: ISDH Laboratories Reporting Requirements (selected slides)

In Indiana, CP-CRE is reportable for both isolates and condition.

- CDR Modification Highlights Actionable Interventions
- Sets a timeline (72 hours) for investigation
- Puts a focus on acute care and long term care
- Prevent spread by encouraging:
  - Contact precautions
  - CDC CRE Toolkit Use
  - Screening for colonization
  - Chlorhexidine bathing



Based on the potential for transmissibility, the Communicable Disease rule focuses on CP-CRE.

In order to narrow the amount of isolates required to submit to the state, the following isolate submission criteria were adopted:

Isolates include organisms that are non-susceptible to at least one carbapenem antibiotic with MIC >=2 µg/ml or zone diameter <=22 mm (<=21 mm for ertapenem)

AND one of the following criteria:

1. Are positive for carbapenemase production by a phenotypic test (e.g. Modified Hodge, or Carba NP).

2. Are non-susceptible to at least three carbapenem antibiotics with MIC >=2 µg/ml or zone diameter <=22 mm (<=21 mm for ertapenem).

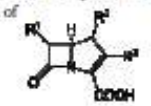
3. Are positive for a carbapenemase gene marker.

Only one isolate that meets these criteria should be submitted if the same organism is repeatedly recovered from the same patient.

AST-based Review and Reporting: A Data-Driven Decision

Review of unnecessary submissions from 2013-2015:

- 98.8% of these submissions could have been prevented with the CDR algorithm:
  - 55.6% by specifying the MIC or Zone Diameter
  - 43.2% by assessing phenotypic carbapenemase production
- Only 58% of these submissions would have been prevented with an alternative submission criteria of resistance to only 2 carbapenems



Structure of carbapenem backbone, wikipedia.org

Three day submission criteria …

(1) CP-CRE
(2) Haemophilus influenzae, invasive disease.
(3) Neisseria meningitidis, invasive disease.
(4) STEC
(5) VRSA
(6) Mycobacterium tuberculosis.
(7) S. pneumoniae invasive disease (<5yo)
(8) Listeria monocytogenes (sterile sites)
(9) Salmonella sp. (clinical specimen)
(10) Shigella sp. (clinical specimen)
(11) Vibrio cholerae (stool or vomit)
(12) Vibrio sp. (clinical specimen)

Day 1 – Receive isolate and subculture

Day 2* – Confirm ID and Perform PCR

- MALDI-TOF MS
- Multiplex PCR [KPC, NDM-1, OXA-48, IMP, VIM]

If Positive, report Finalized result.
- Ex. *Klebsiella pneumoniae*, KPC positive

If Negative – report Preliminary Negative.

Day 3 – CarbaNP on PCR negative specimens

If Positive, send to CDC for further testing.
If Negative, report out as Negative.

ISDH Algorithm

Appendix D: Laboratory Methods of Testing (selected slides)

Other Methods of Carbapenemase Detection: An Overview

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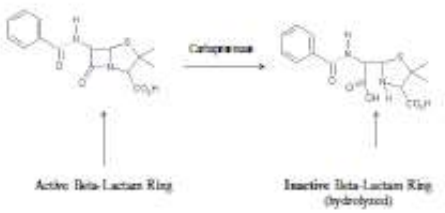
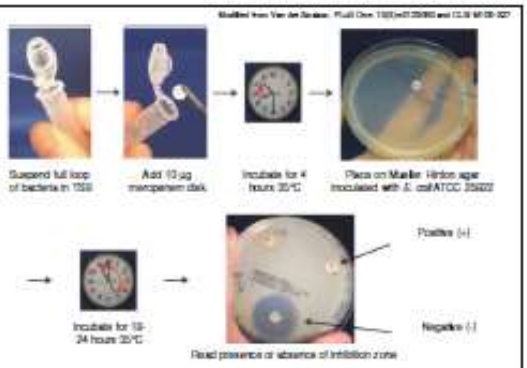
Carbapenemases



Modified Carbapenemase Inhibition Method (mCIM)

Basic Principle of mCIM

1.) Gather materials (swabs, reagents, tubes, plates).
2.) Prepare a suspension of the test organism.
3.) Add a meropenem disk to the suspension. Incubate for 4 hours, 35°C, ambient air.
4.) Prepare a 0.5 McFarland Solution of the *E. coli* carbapenem-susceptible strain (ATCC 25922).
5.) Inoculate plate and allow to dry.
6.) Remove the meropenem disk and place the in the middle of the prepared plate.
7.) Incubate at 35°C, ambient air, 18 hours to overnight.
8.) Inspect plate and interpret.



Interpretation: Positive (6-15mm), Negative (≥19mm), Indeterminate (16-18mm)

Testing Details – mCIM

- **Cost of Test:** ~$1.00 (@ ISDH)
- **Kit Shelf Life:** ~2-3 months (Mueller-Hinton plate)
- **Time to Test:** 18-24 hours
- **Hands on Time:** ~20 minutes
- **Additional Materials:** Mueller Hinton plate, meropenem disk, Tweezers/Forceps, alcohol pad, McFarland standard/turbidity meter, cotton swabs, tubed saline, control organisms