January 2020

CDC AR LABORATORY NETWORK:
Guidance for Testing CRE & CRPA in State and Local Public Health Laboratories

PURPOSE.

State and local public health laboratories were funded through CDC's Epidemiology and Laboratory Capacity Cooperative (ELC) agreement to collect, confirm, and characterize carbapenem-resistant Enterobacteriaceae (CRE) and *Pseudomonas aeruginosa* (CRPA) isolates. These activities help identify isolates that produce a carbapenemase and classify the kind of carbapenemase present. Isolates should be collected from jurisdictional healthcare facilities or clinical labs serving these facilities using a strategy that allows for detecting carbapenemase-producing organisms (CPOs) within the population served by the public health laboratory. State and local public health laboratories will collaborate with state HAI/AR prevention programs and also with the Antibiotic Resistance Lab Network Regional Laboratories. This Guidance document will identify expectations for ELC-funded public health laboratories regarding methods for testing, submitting, reporting, and storage, as well as lab training and proficiencies.

GENERAL CONSIDERATIONS.

When using this guidance, ELC-funded public health laboratories should consider:

- This Guidance recommends options for laboratory testing methods. Methods used by laboratories are not limited to these, but alternative testing choices should yield comparable results, turn-around-time, and accuracy;
- All testing should be implemented in accordance with current Clinical and Laboratory Standards Institute (CLSI) standards and in compliance with Clinical Laboratory Improvement Amendments (CLIA) regulations;
- Laboratories should designate a subject matter expert (SME) to lead laboratory coordination with the state and local health department epidemiology functions, clinical laboratories, and CDC and state HAI/AR prevention programs. This SME should have expertise in antimicrobial resistance detection and clinical diagnostics and have experience in coordination of public health laboratory activities. Up-to-date SME contact information must be made available to CDC, regional AR Lab Network partners, and jurisdictional healthcare facilities;
- Each laboratory shall maintain test results for a minimum of 7 years.

ISOLATE COLLECTION.

Isolate collection strategies:

- CRE collection and characterization:
  - It is recommended that isolate collection be targeted to at least *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, and *Enterobacter* spp. that are resistant to imipenem, meropenem, doripenem,

or ertapenem by standard susceptibility testing methods (i.e., minimum inhibitory concentrations of ≥4 µg/mL for doripenem, imipenem or meropenem or ≥2 µg/mL for ertapenem).
    - If your lab has additional capacity, please test additional genera of CRE, particularly *Providenicia*, *Proteus*, *Morganella*, *Citrobacter,* and *Serratia* (for organisms intrinsically resistant to imipenem, test isolates that are resistant to carbapenems other than imipenem). Evidence indicates that quite a few of these organisms also carry carbapenemases (https://www.cdc.gov/mmwr/volumes/67/wr/mm6723a4.htm).
- CRPA collection and characterization:
    - Submit carbapenem resistant *P. aeruginosa* isolates, defined by resistance to imipenem, meropenem, or doripenem by standard susceptibility testing methods (i.e., minimum inhibitory concentrations of ≥8 µg/mL).
    - To more efficiently detect carbapenemase producers in CRPA, public health laboratories should consider focusing *Pseudomonas aeruginosa* isolate submissions on those that are:
        - Resistant (MIC ≥8 µg/mL) to imipenem, meropenem, or doripenem
          AND
        - Nonsusceptible (i.e., intermediate or resistant MIC ≥16µg/ml) to cefepime or ceftazidime
    - As no AST-based definition is 100% sensitive for identifying CP-CRPA, laboratories should consider accepting CRPA that do not meet ceftazidime and cefepime non-susceptibility requirements on a case-by-case basis when there is a high suspicion for an isolate being a carbapenemase producer. This includes but is not limited to when an isolate is from patient with hospitalization outside the United States or when there is suspicion for transmission (i.e., two or more epidemiologically linked isolates).
    - In order to streamline testing and prioritize resources, CDC recommends public health laboratories focus testing on non-mucoid isolates.
    - Note on pan-nonsusceptible isolates: CRPA isolates that are non-susceptible to all antibiotics tested should routinely be submitted and tested at public health laboratories.

In 2019, CDC evaluated the sensitivity and specificity of many CRPA AST phenotypes to determine which are most predictive of the presence of carbapenemase genes.

The CDC study of AR Lab Network and MuGSI isolates determined that this phenotypic definition (listed above) had >90% sensitivity for detecting CP-CRPA among CRPA. Nearly two-thirds of the CRPA submitted to clinical laboratories were susceptible to cefepime and ceftazidime. Therefore, using this definition is estimated to reduce the volume of CRPA isolates submitted for carbapenemase testing by approximately 67% and improve labs' efficiency of detecting CP-CRPA among CRPA.

Laboratories that currently receive a subset of CRPA isolates from clinical labs should aim to maintain current CRPA testing volumes when implementing the new definition. Given that the new definition will likely decrease the number of submissions from each currently participating laboratory, public health labs can maintain testing volume by accepting a greater proportion of CRPA identified by currently participating clinical laboratories or by expanding to include submissions from additional clinical laboratories.

Testing and Recruitment Strategies:
If possible, test all CRE and/or CRPA isolates. If states do not have the capacity to test all CRE and/or CRPA, consider prioritizing testing based on:

- **Public health lab testing priorities:** Workflows for CRE and CRPA should ensure that testing priorities are achieved.
    1. Mechanism testing for carbapenemase genes
    2. Carbapenemase production testing
    3. Organism identification/confirmation
    4. Antimicrobial susceptibility testing
        - Note: all suspected pan-NS isolates must be tested

- **Recruitment and sampling:** Test isolates from high risk healthcare facilities and/or populations within the state. At a minimum, isolates should be collected from clinical laboratories that serve both short-term acute care hospitals and long-term acute care hospitals. If possible, isolate collection should be extended to include labs serving other types of healthcare facilities (e.g., long-term care facilities, outpatient clinics).
    - Please refer to the **Supplemental Guidance: Targeting facilities and clinical laboratories for AR Lab Network testing of CRE and CRPA** for more information on targeting isolate submission in your clinical lab network (located on Sharefile under 2019 ELC documents in the G1 G2 supplementary guidance folder).
    - A sampling scheme that is representative and is based on priorities identified using local epidemiology.

- **Streamlining testing using clinical lab results**: Clinical lab testing results may be used to guide workflow prioritization to help reduce public health lab testing burden and avoid duplication of effort. For example:
    - Always request to test any isolate that is non-susceptible to all drugs tested by clinical lab.
    - If any carbapenemase testing results (i.e., phenotypic detection of carbapenemase production or genotypic mechanism detection) are available from submitting clinical labs:
        - consider requesting only isolates that are carbapenemase production testing positive,
        - consider requesting only Cepheid CarbaR positive isolates or ensure they are reporting positive test results to public health department, and/or
        - If clinical lab is conducting both carbapenemase and mechanism testing, consider requesting only isolates that are carbapenemase production testing positive and Cepheid negative, while ensuring pcr-positive test results are reported to public health department.

LABORATORY TESTING.

Protocols posted on ShareFile site

1. **Species identification**
    - Species should be confirmed on all Enterobacteriaceae and *Pseudomonas* received using one of the methods listed below:
        - MALDI-TOF mass spectrometry
        - Automated instruments (e.g., VITEK 2, MicroScan, Phoenix, MIDI, etc.)
        - Biochemical tests

2. **Antimicrobial susceptibility testing (AST) to confirm phenotypic detection of CRE and CRPA:**
    - All isolates should be tested for susceptibility to carbapenems to confirm resistance. Methods used in the lab should ideally complement rather than duplicate methods used by the submitting laboratory (i.e., most submitting clinical labs use automated AST devices like a Vitek 2, MicroScan, or Phoenix). Public health labs should use one of the methods listed below:
        - Disk diffusion
        - Etest – [Note: colistin should be tested by broth microdilution only due to inaccuracies with Etest detecting resistance at lower MICs]
        - Broth microdilution using frozen or dried panels

3. **Drugs for AST:**
    - At minimum, isolates should be tested against all drugs listed in Table 1.
    - Note: CDC is no longer recommending that public health laboratories conduct colistin susceptibility testing. Beginning in 2020, CLSI colistin interpretative criteria for Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter* species will be intermediate or resistant (no susceptible category).
        - Testing for *mcr* genes has also been removed from AR Lab Network priorities. See below for information.

Table 1. Drugs used to confirm and further characterize CRE and CRPA

Drug class	CRE	CRPA
Carbapenems	2 carbapenems (ertapenem and either imipenem, doripenem or meropenem)	2 carbapenems (selected from imipenem, doripenem and meropenem)
Cephems	ceftazidime, ceftriaxone[a], and cefepime	ceftazidime and cefepime
B-lactam/B-lactamase inhibitor combinations	NA	piperacillin-tazobactam
Monobactams	aztreonam	aztreonam

[a]Other third-generation cephalosporin(s) may be substituted for ceftriaxone

4. **Phenotypic methods to detect carbapenemase production:**
    - These tests can be used to determine if the isolate produces a carbapenemase, but do not determine which carbapenemase is present. The test options are:
        - Modified Carbapenem Inactivation Method (mCIM) -- preferred method
        - CarbaNP assay including commercially available CarbaNP tests

5. **Molecular Detection**

Isolates should be tested for the following targeted carbapenemase genes

Table 2. Molecular targets for CRE and CRPA.

	CRE	CRPA
PCR Targets	*bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-48-like}$, *bla*$_{IMP}$, *bla*$_{VIM}$	*bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{IMP}$, *bla*$_{VIM,}$

Table 3. List of recommended PCR detection methods

Test Name	Type	Source	Targets	Instrumentation
Real-time PCR KPC/NDM	PCR	CDC	bla_{KPC}/bla_{NDM}	ABI 7500
Real-time PCR OXA-48	PCR	CDC	$bla_{OXA\text{-}48\text{-}like}$	ABI 7500
Real-time PCR VIM	PCR	CDC	bla_{VIM}	ABI 7500
Real-time PCR IMP*	PCR	CDC	bla_{IMP}	ABI 7500
ARM-D	PCR	Streck	bla_{KPC}, bla_{NDM}, bla_{OXA}, bla_{VIM}, bla_{IMP}*, $bla_{CTX\text{-}M\text{-}14}$, $bla_{CTX\text{-}M\text{-}15}$, bla_{DHA},	ABI 7500
X-pert Carba-R	PCR	Cepheid	bla_{KPC}, bla_{NDM}, OXA-48-like genes, bla_{VIM}, $bla_{IMP\text{-}1}$# group	GeneXpert®
Nanosphere gram-negative blood culture (BC-GN)	PCR	Luminex	bla_{KPC}, bla_{NDM}, bla_{OXA}, bla_{VIM}, $bla_{IMP\text{-}1}$, $bla_{CTX\text{-}M}$	Verigene

* This assay encompasses all circulating IMP variants in US.

This assay encompasses only $bla_{IMP\text{-}1}$ group variants.

Note: Detection of mcr genes has been de-escalated from AR Lab Network priorities, and therefore testing of *mcr* genes is no longer requested by DHQP. If labs prefer to continue testing, please still report positive findings to CDC for situational awareness.

REQUIRED REPORTING.

1. **Labs will report testing results back to the submitting institutions within 2 working days via secure communications** (i.e., established protocols that may include utilization of standardized data exchange platforms and systems, secure fax, or encrypted e-mail), with a disclaimer that testing results:
    - Should be used to support infection prevention measures.
    - Should not be a substitute for diagnostic procedures or used to guide clinical decisions.

Labs will report any isolates with defined AR Lab Network Alert values to their state HAI coordinator and CDC within 1 day via using REDCap "ARLN Alert" database (https://rdcp.cdc.gov)) to facilitate immediate public health actions. All ARLN Alerts sent will be routed to DHQP laboratory and outbreak response groups.

Findings that should trigger immediate alerts include:

- **Pan-resistance.** An isolate that is non-susceptible to all drugs tested to date, including those tested by the submitting clinical laboratory and your public health lab (any organism).
- **Non-KPC carbapenemase in Enterobacteriaceae**
- **Carbapenemase-producing CRPA.** *Pseudomonas aeruginosa* positive for carbapenemase production and/or positive by PCR for bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP} genes. *Pseudomonas aeruginosa* has not been found to harbor $bla_{OXA\text{-}48\text{-}like}$ and therefore routine testing for $bla_{OXA\text{-}48\text{-}like}$ is not recommended. However, if *P. aeruginosa* with $bla_{OXA\text{-}48\text{-}like}$ is identified, an alert should be sent.

- **Class A or B carbapenemase-gene positive *Acinetobacter baumannii*.** CRAB with *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$ genes detected. Note that *A. baumannii* has not been found to harbor *bla*$_{OXA-48-like}$ and therefore routine testing for *bla*$_{OXA-48-like}$ is not recommended. However, if *A. baumannii* with *bla*$_{OXA-48-like}$ is identified, an alert should be sent.
- **Class D carbapenemase-gene positive *Acinetobacter baumannii*.** CRAB with *bla*$_{OXA-23}$, *bla*$_{OXA-24/40}$, *bla*$_{OXA-58}$, carbapenemase in *Acinetobacter baumannii*. If these organisms are <u>not</u> endemic or routinely identified in the region, an alert should be sent to the HAI coordinator and to CDC. For regions where these organisms are endemic or routinely identified, an alert does not need to be sent to CDC. Each jurisdictional public health laboratory should discuss reporting of these organisms with their HAI coordinator. In general, CRAB producing oxacillinase carbapenemases are rare, highly resistant, and can spread in healthcare facilities; therefore, sending alerts to the HAI coordinator will help ensure healthcare facilities are aware and may help stop transmission.
- **Suspected novel carbapenemase.** CRE or CRPA positive for carbapenemase production by phenotypic methods and negative by PCR to *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-48-like}$, (for CRE) *bla*$_{IMP}$, *bla*$_{VIM}$.
  - Please **<u>exclude</u>** *Serratia spp.* resistant to carbapenems and susceptible to 3rd generation cephalosporins. This resistance profile indicates an *bla*$_{SME}$ gene, not novel resistance.
  - Please **<u>exclude</u>** isolates that are cefotaxime, ceftriaxone, and ceftazidime intermediate/resistant but cefepime susceptible. This AST profile is consistent with false positive mCIM+ results, likely because of high levels of AmpC beta-lactamase(s).
  - Please **<u>include</u>** isolates that are resistant to carbapenem(s) but susceptible to cefotaxime, ceftriaxone, and ceftazidime. This AST profile is indicative of a possible *bla*$_{IMI\ or}$ *bla*$_{NMC}$ gene (both class A carbapenemases).
- **KPC-positive Enterobacteriaceae.** Discuss reporting KPC-CRE with your HAI coordinator. In most of the United States, *bla*$_{KPC}$ meets criteria for a Tier 2 (not regularly found in the region) or Tier 3 (identified before in the region but not endemic) organism for which each identification requires a public health response, as outlined in the **<u>Interim Guidance for a Health Response to Contain Novel or Targeted Multidrug-resistant Organisms (MDROs)</u>**. Your local epidemiology should inform your response activities. If *bla*$_{KPC}$ is not endemic in your jurisdiction or in the region of your jurisdiction where it was detected, please also send an alert to CDC.
- **Carbapenemase detected during colonization screening.** Specimens testing positive for *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-48-like}$, *bla*$_{IMP}$, *bla*$_{VIM}$ genes detected as a result of colonization screenings (e.g., Cepheid). Please also include the state lab ID of the index isolate(s) that initiated the screening, if known (any organism). For any organism isolated from a screening swab, please send an updated alert with testing results.
- **NEW! Aztreonam-avibactam MIC ≥8/4 µg/mL.** An isolate that was tested as part of the Expanded Antimicrobial Susceptibility Testing for Hard-to-treat infections (Enterobacteriaceae). (See ExAST guidance for more information). Alerts will be generated by testing Regional Lab who will alert state/local jurisdictions when testing is performed and when an alert value isolate is detected.
- ***mcr*-type resistance**. Detection of any *mcr* gene in by PCR or WGS (any organism).

2. **Labs will report CRE and CRPA testing results to CDC monthly, by the 10th of each month for the previous month's testing.** This report will be submitted through the APHL AIMS portal. If a lab is not yet in production for AIMS submission, monthly summaries may be submitted using the monthly reporting form for

CRE and CRPA (available on the Share File site)**.** CDC and APHL are building infrastructure to receive data in HL7 messages. The goal for the next year is to working closely with states to transition sending data using HL7 format in a more timely frequency.

- Send reports to Jennifer Huang, at uzo0@cdc.gov
- Recommend sending reports to your submitting facilities (in aggregate or as lab-specific line lists) to encourage continued participation and auditing of isolates sent.

STORAGE AND SENDING ISOLATES TO AR REGIONAL LAB.

1. Labs should **store all CRE and CRPA isolates with confirmed carbapenem resistance** (one isolate per patient) for a minimum of 2 years.

2. **When a novel and/or unusual resistance mechanism** (i.e., discordant results) is detected, the isolate and required data should be submitted to the designated AR Lab Network Regional Lab within 1 working day. If the clinical lab is conducting carbapenemase production testing using mCIM/CarbaNP and molecular testing using Cepheid Carba-R, there is no need to repeat testing at the PHL, send the isolate directly to the Regional Lab.
    - CDC may request that some isolates be submitted to CDC. On request, isolates and required data should be submitted to CDC within 1 working day.
3. **For suspected pan-r isolates** that are non-susceptible to all drugs tested at public health lab and clinical lab, contact CDC for appropriate routing of the isolate (to CDC or Regional Lab) based on drugs tested, organism and whether a carbapenemase was identified. If the clinical lab tests against an extensive panel of drugs and the drugs at the PHL are duplicative, there is no need to repeat testing.

LABORATORY TRAINING AND SAFETY

1. **Training:** Training will be coordinated by APHL and CDC. Staff will receive necessary training from their AR Regional Labs. Labs can also contact CDC with any technical assistance needs.
2. **Safety:** All CLIA-mandated laboratory safety protocols must be followed for all testing.

CONTACT INFORMATION.

For questions or further information, please contact Dr. Sarah Malik (vgg9@cdc.gov) or the CDC AR Lab Network inbox at ARLN@cdc.gov.