OKLAHOMA State Department of Health

Public Health Laboratory

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Carbapenem-resistant Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter* spp.

Use

Confirmation and characterization of carbapenem-resistant Enterobacterales (CRE), *Pseudomonas aeruginosa* (CRPA), and *Acinetobacter* spp. (CRA) isolates from Oklahoma healthcare facilities for epidemiological purposes. These tests are intended as an aid for infection control of carbapenem-non-susceptible organisms in healthcare settings. They are not intended to guide or monitor treatment for carbapenem-non-susceptible bacterial infections.

Per the Oklahoma Administrative Code, Title 310 Chapter 515-1-8, "*…pure isolates of these organisms shall be sent to the OSDH Public Health Laboratory for additional characterization, typing or confirmation within two (2) working days (Monday through Friday, state holidays excepted) of final identification or diagnosis*".

Clinical Significance

Carbapenemase-producing *Enterobacterales*, *P. aeruginosa* and *Acinetobacter* spp. are of growing public health concern. They are often resistant to all beta-lactam agents and can be co-resistant to multiple classes of other antimicrobial agents. Identifying isolates that produce carbapenemases and classifying the kind of carbapenemase present is important in preventing their spread.

Further background information, fact sheets, statistics and educational resources may be found at the OSDH Infectious Disease Services website.

Methodology

Isolates of Enterobacterales, *P. aeruginosa,* and *Acinetobacter* spp. are initially subjected to MALDI-ToF-mass spectrometry and/or biochemical testing to confirm species. Successfully identified isolates subsequently undergo a variable combination of Modified Carbapenemase Inactivation Method (mCIM) to confirm phenotypic carbapenem resistance, Antimicrobial Sensitivity Testing (AST) using a broth microdilution method (MicroScan WalkAway System) and molecular detection of *KPC*, *NDM*, *VIM*, *IMP* and *OXA-48* antimicrobial-resistance genes (Cepheid Xpert® Carba-R IVD), according to CDC's *Guidance for Testing Carbapenem-Resistant Enterobacterales, Pseudomonas aeruginosa,* and *Acinetobacter baumannii Isolates from Human Specimens in State and Local Public Health Laboratories*. *Acinetobacter* spp. isolates are referred to the Antimicrobial Resistance Laboratory Network (ARLN) Regional Laboratory or CDC for detection of *KPC*, *NDM*, *VIM*, *IMP*, and *OXA* variant genes.

Specimen Type

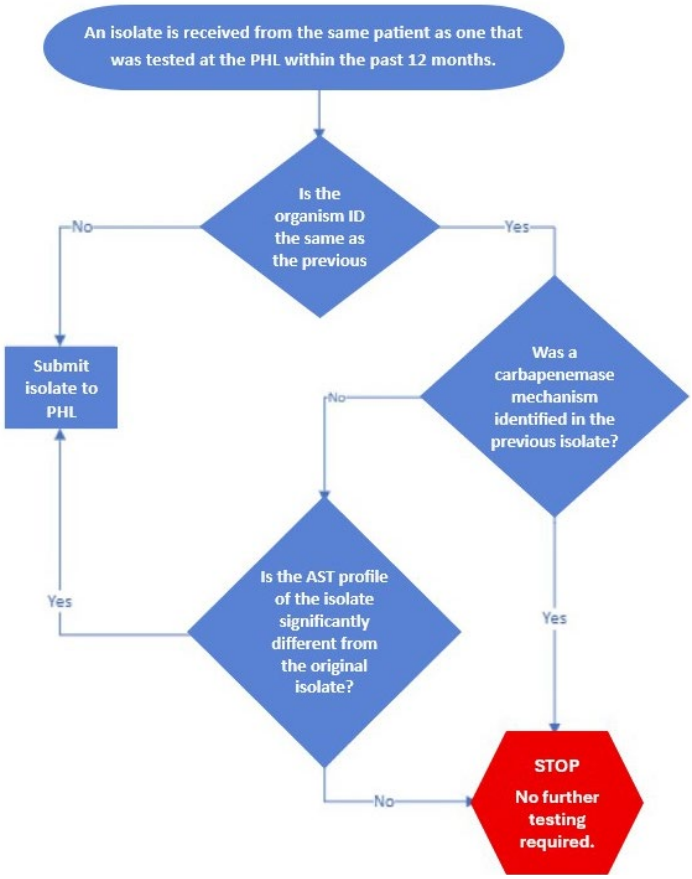
Organism	Definition	Submission Requirements
Carbapenem Resistant Enterobacterales (CRE)	Resistant to ertapenem, imipenem, or meropenem using current CLSI breakpoints (i.e., minimum inhibitory concentration (MIC) of ≥ 2 µg/mL for ertapenem and ≥ 4 µg/mL for imipenem or meropenem)	Pure isolate of confirmed or suspected CRE grown for 18-24 hours on Trypticase Soy Agar (TSA) with 5% Sheep's Blood (BAP)
Carbapenem Resistant *Pseudomonas aeruginosa* (CRPA)	Resistant to imipenem or meropenem using current CLSI breakpoints (i.e. minimum inhibitory concentrations of ≥ 8 µg/mL) **AND**	Pure isolate of confirmed or suspected CRPA grown for 18-24 hours on TSA BAP.

Organism	Definition	Submission Requirements
	Nonsusceptible (i.e., intermediate or resistant MIC ≥ 16 µg/mL) to cefepime or ceftazidime, or, resistant to ceftolozane/tazobactam (MIC ≥ 16/4 µg/mL) **Note 1**: *If facility does not use non-standard AST or does not test all of the requested drugs, PHL will review the provided AST results and perform testing on a case-by-case basis.* **Note 2**: *Given the difficulties in testing, CDC does not recommend testing isolates from cystic fibrosis patients or mucoid isolates.*	
Carbapenem Resistant *Acinetobacter baumannii* (CRAB)	*Acinetobacter* spp. resistant to imipenem or meropenem using current CLSI breakpoints (i.e. minimum inhibitory concentrations of ≥ 8 µg/mL) ***Note:*** A. radioresistans *is known to harbor a chromosomal carbapenemase gene and should not be submitted for further testing.*	Pure isolate of confirmed or suspected CRAB grown for 18-24 hours on TSA BAP
Pan Not-Susceptible CRE or Pan-Resistant CRPA/CRAB	CRE: CRE-not-susceptible (intermediate or resistant) to all drugs tested at the submitting clinical laboratory CRPA/CRAB: CRPA or CRAB resistant to all drugs tested at the submitting clinical laboratory	Pure isolate of confirmed or suspected CRAB grown for 18-24 hours on TSA BAP

Minimum Volume/Size
Minimum of 1 agar plate or slant with visible growth

Collection Instructions
Primary specimens should be collected according to the submitting institution's standard procedure.

- Submit pure isolates on a BAP or MacConkey agar plate.
- For isolates submitted from the same patient within the past 12 months that are of the same species:
  - If a carbapenemase mechanism was identified in the original isolate, do not submit the new isolate.
  - If a carbapenemase mechanism was not identified in the original isolate, but the AST profile is not significantly different, do not submit the new isolate.
  - If there is a special need to test or a suspicion of acquiring a new mechanism, please contact the PHL at (405) 564-7750 for approval prior to submission.
- Per What to Report (oklahoma.gov) (OAC 310:515-1-8), specimens must be submitted within two (2) working days (Monday through Friday, State holidays excepted) of final identification or diagnosis.
- Incubate all isolates in appropriate atmosphere for



18-24 hours prior to shipping.
- Non-suppressed Antimicrobial Susceptibility Testing (AST/MIC) reports must be submitted with the isolate and OSDH PHL *Test Requisition Form* (ODH 419).

Common Causes for Rejection
- Media expired
- No growth
- Non-viable specimen
- Frozen specimen
- Specimen leaked in transit
- Mucoid CRPA isolates

Shipping
Ship isolates that meet the submission requirements listed in the *Specimen Type* table above at room temperature on appropriate media.

Turn-around Time
Test results are reported within 5 working days from receipt for CREs and CRPAs, and within 7 working days from receipt for CRAs. Referral of isolates to the ARLN Regional Laboratory or CDC for further characterization may delay the availability of final results.

Reference Range
- Bacterial Isolate: Isolate could not be identified to species level; carbapenem-resistance testing not performed
- mCIM: Negative
- Xpert CarbaR PCR: [KPC, NDM, VIM, IMP and/or OXA-48] or no carbapenem-resistance genes detected

Reportable Results
Bacterial Isolate Identification, mCIM Carbapenemase, and Xpert® Carba-R PCR results are reported to the submitter; however, these results are **intended solely for surveillance purposes and must not be used for clinical management decisions**. AST results of the MicroScan WalkAway System are not reported to the submitter.
- Bacterial Isolate, Identification/Serotyping/Confirmation:
  - Carbapenem-resistant *Acinetobacter* species specimen has been received.
  - *Enterobacter cloacae* complex
  - *Escherichia coli*
  - *Klebsiella aerogenes*
  - *Klebsiella oxytoca/Raoultella ornithinolytica*
  - *Klebsiella pneumoniae*
  - *Klebsiella veriicola*
  - *Morganella morganii*
  - *Proteus mirabilis*
  - *Providencia rettgeri*
  - *Providencia stuartii*
  - *Pseudomonas aeruginosa*
  - *Serratia marscens*
- mCIM:Carbapenemase:
  - Positive

    - Negative
    - Indeterminate
- Xpert® Carba-R PCR:
    - [KPC, NDM, VIM, IMP and/or OXA-48] gene(s) detected
    - No carbapenem-resistance genes detected
    - Invalid

Isolates demonstrating a potentially new carbapenemase variant or novel mechanism of resistance or isolates that produce discordant results may be forwarded to an ARLN Regional Laboratory or the CDC for further testing. These results are not reported to the submitter.

Interpretation

- Isolates demonstrating carbapenem resistance by phenotypic mCIM with a positive Xpert® Carba-R result for one or more resistance genes are confirmed carbapenem-resistant due to the presence of one or more carbapenemases.
- Isolates demonstrating carbapenem resistance by phenotypic mCIM with a negative Xpert® Carba-R result for resistance genes are confirmed carbapenem-resistant, potentially due to the presence of a new variant carbapenemase or other novel mechanism of resistance.
- Isolates demonstrating carbapenem sensitivity by phenotypic mCIM are confirmed carbapenem-sensitive.

Limitations/Interferences

- Discordant results are expected between the different methods.
    - Hydrolysis of carbapenem by carbapenemases is the most common mechanism of resistance for this class of antibacterial agents but other mechanisms of resistance occur and may not be detected by PCR.
    - A carbapenemase may be weakly expressed producing a negative phenotypic test or the gene may be present in low copy numbers producing a negative PCR.
    - Phenotypic antimicrobial susceptibility tests demonstrate variable sensitivities and specificities and use different combinations of antibiotics and inhibitors.
- Isolates that are not successfully identified to the genus/species level will not be tested.
- Isolates that are negative for mCIM will not be tested by the Xpert® Carba-R assay.

CPT Code

CPT codes will vary depending on organism identified and methods used.

Notes

The mCIM is a laboratory-developed test; performance characteristics have been validated and determined to be suitable for diagnostic purposes by the OSDH PHL. The Bruker MALDI-ToF-MS Biotyper and Xpert® Carba-R assay are approved for *in vitro* diagnostic use by the U.S. Food and Drug Administration.