North Carolina CRE Laboratory Task Force

Carbapenem-Resistant Enterobacteriaceae (CRE) Screening and Confirmatory Testing Recommendations in North Carolina

2018

Consensus guidelines developed by the
North Carolina Carbapenem-Resistant Enterobacteriaceae Laboratory Task Force
in association with the North Carolina Surveillance for Healthcare
Associated and Resistant Pathogens Patient Safety
Program and the North Carolina Laboratory Response Forum

North Carolina Carbapenem-Resistant Enterobacteriaceae Laboratory Task Force Members:

Elizabeth Palavecino, **Wake Forest University Baptist Medical Center;** Gerald Capraro, **Carolinas Pathology Group, Atrium Health;** Heather Dubendris, **CDB/NCDPH**; James Lewis, **CDB/NCDPH;** Kevin Hazen, **Duke University;** Robert Sautter, **RL Sautter Consulting LLC.;** Shermalyn Greene, **SLPH/NCDPH**

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About the Authors:

Elizabeth Palavecino Elizabeth Palavecino, M.D. is the Director of the Clinical Microbiology Laboratory at Wake Forest Baptist Medical Center and Laboratory Director at Forsyth County Laboratory of Public Health in Winston Salem, NC.

Gerald Capraro: Dr. Capraro is board certified in by the American Board of Medical Microbiology and serves as the Medical Director of the Clinical Microbiology Laboratory for Atrium Health (formerly, Carolinas HealthCare System).

Heather Dubendris Heather Dubendris is an epidemiologist with the North Carolina Division of Public Health, Communicable Disease Branch, Surveillance for Healthcare Associated and Resistant Pathogens Patient Safety Program

James Lewis Dr. James Lewis is the medical director of the North Carolina Division of Public Health, Communicable Disease Branch, Surveillance for Healthcare Associated and Resistant Pathogens Patient Safety Program

Kevin Hazen Kevin C. Hazen, Ph.D., D(ABMM), FAAM, FIDSA is a Director of Clinical Microbiology and Professor of Pathology where he serves as Section Head of Bacteriology, Mycobacteriology and Molecular Microbiology and previously was Medical Director of Clinical Microbiology at the University of Virginia where he engaged in collaborative research on transmission of CRE in in-patient populations.

Robert Sautter Robert L. Sautter, PhD HCLD/CC (ABB) is the principle for RL Sautter Consulting LLC in Lancaster, SC and past affiliations include; Director of Microbiology and Point of Care for the Carolinas Pathology Group, Charlotte and also Harrisburg PA, Director and Technical Director for the Mecklenburg County Health Laboratory, Director of the School of Medical Technology for Harrisburg Hospital, Assistant Professor of Pathology for Penn State Hershey Med. Ctr.

Shermalyn Greene Shermalyn R. Greene, PhD is the Manager of the Molecular Diagnostics and Molecular Epidemiology Program at the North Carolina State Laboratory of Public Health in Raleigh, NC.

BACKGROUND

Antibiotic management of infections caused by gram-negative bacteria is an ongoing challenge faced by physicians as these bacteria can express various antibiotic resistance mechanisms, including those that affect uptake of a drug (porin mutations), retention of the drug (efflux pump modifications), drug target site mutations and production of drug inactivation enzymes. One of the most common mechanisms of resistance used by gram negative bacteria against beta-lactam drugs is the production of beta-lactamases. In the past, these beta-lactamases were limited in their specificity, but mutations in the genes encoding the beta-lactamases have led to enzymes with a wider spectrum of specificity, such as the extended spectrum beta-lactamases (ESBLs). Emergence of ESBLs limited the available antibiotic armamentarium available to physicians, but carbapenems remained effective against most organisms until recently. With the increased use of carbapenems, emergence of organisms expressing beta-lactamases active against carbapenems (carbapenemases) was only a matter of time.

Carbapenemase genes have been detected in a wide variety of bacteria and are given various designations, such as KPC (*Klebsiella pneumoniae* carbapenemase), NDM (New Dehli metallo-β-lactamase), OXA (oxacillin hydrolyzing β-lactamase), GES (Guyana extended spectrum β-lactamase), VIM (Verona integron-encoded metallo-β-lactamase), SME (*Serratia marcescens* enzyme) and CTX-M (Cefotaximase-München β-lactamase). These abbreviated names may or may not have any biological relevance to the activity of the specific carbapenemase. Carbapenemase genes may reside on chromosomes or on plasmids. Additionally, expression of carbapenemases may be continuous at low or high-levels or may be inducible. Some carbapenemases, as well as additional drug resistance genes, may be located within integrons and transposons, allowing the genes to be inserted into plasmids and chromosomes.

Within the United States, the most common plasmid mediated carbapenemase is the *Klebsiella pneumoniae* carbapenemase (KPC), encoded by the *bla*KPC gene. Interestingly, KPC was first described in an isolate of *K. pneumoniae* collected from a patient in 1996 in North Carolina. KPC-producing bacteria have now been detected across the United States and are increasingly identified as a cause of healthcare-associated infections. While originally described in *K. pneumoniae*, *bla*KPC has been detected in other species of *Klebsiella* as well as in other genera of Enterobacteriaceae and in *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and others.

Infections with carbapenem-resistant Enterobacteriaceae (CRE) are associated with a higher attributable mortality than infections with Enterobacteriaceae that are susceptible to carbapenems. Additionally, once CRE are established within a healthcare facility, eradication becomes challenging. For these reasons, microbiology laboratories in concert with infection prevention programs must be vigilant in their efforts to detect these organisms before the organisms become established within the healthcare facility. Such efforts include, but are not necessarily limited to, detection of CRE in clinical specimens and when appropriate, surveillance to assess the prevalence of CRE within a facility.

In 2012 the Centers for Disease Control and Prevention (CDC) published a CRE prevention toolkit. The contributions of clinical laboratories is critical to the success of CRE prevention. The North Carolina Division of Public Health (NCDPH) convened a taskforce of Clinical Microbiology and Infection Prevention experts to develop guidelines for detection of CRE by clinical laboratories within the state.

The first set of guidelines were published in 2014. Since then a number of laboratory and programmatic changes have necessitated an update to the guidelines including: CDC established a new, more inclusive definition in 2015 (with increased sensitivity for detection of carbapenemase producing CRE (CP-CRE) strains), phenotypic testing methods have improved and commercial methods for molecular characterization of carbapenemases and resistance testing have become available. Additionally, the Council of State and Territorial Epidemiologists passed a position statement in 2017 to make CP-CRE nationally notifiable; CP-CRE became nationally notifiable in 2018.

The value of statewide CP-CRE reporting is enhanced by the establishment of standardized definitions and practices for detection of these organisms. This update provides laboratory guidelines to ensure streamlined detection and surveillance of CRE in North Carolina while considering that laboratories vary in their capacity to detect and characterize CRE. This update, similar to the guidelines issued in 2014, does not address CRE surveillance strategies, but healthcare institutions should be cognizant of the significant role surveillance can play in limiting spread of carbapenemase producing organisms.

STATEMENT OF DEFINITIONS AND PURPOSE

PURPOSE

This document contains recommendations for the laboratory detection of CRE and CP-CRE according to the methodology and breakpoints used for testing and interpreting antimicrobial susceptibility results. These guidelines are to be applied to laboratories to help guide infection prevention and public health response. These guidelines were developed by an expert panel based on CDC, Clinical Laboratory Standard Institute (CLSI), and the Food and Drug Administration (FDA) guidelines and recommendations.

DEFINITIONS

An increase in the minimal inhibitory concentrations (MICs) for carbapenems does not necessarily reflect production of carbapenemases, as other mechanisms such as porin mutations, increased efflux pump activity, or presence of enzymes other than carbapenemases can also produce resistance to carbapenems. Independently of the mechanism of resistance, infections caused by carbapenem resistant organisms are difficult to treat. Table 1 summarizes the terminology and the acronyms commonly used to describe carbapenem resistance in Enterobacteriaceae.

Table 1: Summary of carbapenem resistance terminology

Term	Acronym	Definition	Laboratory Detection
Carbapenem Resistant Enterobacteriaceae	CRE	Organisms from the family Enterobacteriaceae that are resistant to carbapenems regardless of the mechanism	Organism is resistant to any of the carbapenems by susceptibility testing methods using current breakpoints
Carbapenemase producing Carbapenem-Resistant Enterobacteriaceae	CP-CRE	Organisms from the family Enterobacteriaceae that are resistant to carbapenems due to carbapenemase production	A positive result from a carbapenemase test (phenotypic or molecular test)
Non Carbapenemase producing-Carbapenem resistant Enterobacteriaceae	Non CP-CRE	Organisms from the family Enterobacteriaceae that are resistant to carbapenems due to mechanisms other than carbapenemase production.	Organism is resistant to any of the carbapenems by susceptibility testing methods using current breakpoints and negative for carbapenemase production or mechanism on phenotypic or molecular tests

* Information modified from the following reference: CLSI AST Newsletter Volume 2, Issue 1, June 2017.

Identification of CP-CRE

Identification of the presence of carbapenemases or other mechanisms of resistance is not necessary when deciding if a carbapenem might be a therapeutic option for a patient. However, for epidemiologic or infection prevention purposes, laboratories may require identification of carbapenemase-producing Enterobacteriaceae. Because carbapenemase-encoding genes are often located on plasmids, this type of resistance is much more likely to spread. Therefore, the distinction between carbapenem-resistance mediated by carbapenemases and resistance mediated by other mechanisms is important for infection prevention. CP-CRE are a subset of all CRE and have been targeted for prevention because they have the ability to spread rapidly and can cause infections that are associated with high mortality rates[1].

For the purpose of this document, CRE are defined as Enterobacteriaceae species from any site that are:

- Resistant to ertapenem, imipenem, meropenem or doripenem by current Clinical Laboratory Standard Institute (CLSI) breakpoints (first described in M100-S21)

OR

- Positive for carbapenemase production by a phenotypic test (e.g., the Modified Carbapenemase Inactivation Method [CIM])

OR

- Positive for carbapenemase genetic determinants by molecular methods

Reporting to the North Carolina Division of Public Health: While many Enterobacteriaceae may be CRE, *Escherichia coli (E. coli)*, *Enterobacter* spp, and *Klebseilla* spp. are most commonly identified and to date are the only CRE designated as nationally notifiable. In North Carolina, identification of CRE from a clinical specimen associated with either infection or colonization, including all susceptibility results and all phenotypic or molecular test results are reportable as of October 1, 2018. For the purposes of reporting, Carbapenem-Resistant Enterobacteriaceae (CRE) are defined as:

> *(a) Enterobacter* spp., *E.coli* or *Klebsiella* spp. positive for a known carbapenemase resistance mechanism or positive on a phenotypic test for carbapenemase production; or
> (b) *Enterobacter* spp., *E.coli* or *Klebsiella* spp. resistant to any carbapenem in the absence of carbapenemase resistance mechanism testing or phenotypic testing for carbapenemase production

More information on reporting requirements in NC can be found in appendix B.

References:

1. CDC. Antibiotic resistance threats in the United States, 2013. Atlanta, GA: US Department of Health and Human Services, CDC; 2013. Available at http://www.cdc.gov/drugresistance/threat-report-2013.

BREAKPOINTS FOR CARBAPENEMS

The CLSI lowered the breakpoints for the carbapenems in Enterobacteriaceae; these new (current) breakpoints were published in the M100 document in 2011 (1). Table 2 lists the current CLSI disk diffusion breakpoints for carbapenems. Table 3 lists the previous and current minimum inhibitory concentration (MIC) breakpoints for carbapenems (2).

Table 2. Disk Diffusion Zone Diameter Breakpoints (mm) for Carbapenems for Enterobacteriaceae.

	CLSI Breakpoints* (described in M100-S28; first described in M100-S21)		
	Zone Breakpoints Criteria (mm)		
Drug	**Suseptible (S)**	**Intermediate (I)**	**Resistant (R)**
Doripenem	≥23	20-22	≤19
Ertapenem	≥22	19-21	≤18
Imipenem	≥23	20-22	≤19
Meropenem	≥23	20-22	≤19

* Laboratories using disk diffusion can implement the current breakpoints immediately

Table 3. MIC Breakpoints (µg/ml) for Carbapenems for Enterobacteriaceae.

	CLSI Breakpoints (described in M100-S20)			**CLSI Breakpoints*** (described in M100-S28); first described in M100-S21		
	MIC Breakpoints (µg/mL)			**MIC Interpretive Criteria (µg/mL)**		
Drug	**S**	**I**	**R**	**S**	**I**	**R**
Doripenem	-	-	-	≤1	2	≥4
Ertapenem	≤2	4	≥8	≤0.5	1	≥2
Imipenem	≤4	8	≥16	≤1	2	≥4
Meropenem	≤4	8	≥16	≤1	2	≥4

*Laboratories using automated susceptibility systems must have completed an internal validation before implementing the current (first described in M100-S21) CLSI breakpoints if the system has FDA approved breakpoints for the panel the laboratory is using that are different from the current (first described in M100-S21) CLSI breakpoints.

For accurate determination and detection of organisms resistant to carbapenems, laboratories are strongly encouraged to implement current breakpoints (first described in M100-S21) for each of the carbapenems as soon as possible. A laboratory that is not using the current breakpoints can implement these current CLSI carbapenem breakpoints immediately through one of two ways:

- Use of the disk diffusion method

OR

- If using automated antimicrobial susceptibility test (AST) systems, conducting an appropriate internal validation study that includes the lower dilutions needed to apply the breakpoints

Laboratories should contact the manufacturer to determine how the commercial AST system's software will be able to accommodate the revised breakpoints.

Before implementation of the current CLSI breakpoints for carbapenems on an AST system, the laboratory MUST perform validation as required by Clinical Laboratory Improvement Amendments (CLIA) if the system has FDA approval for breakpoints that are different from those of CLSI. The Infectious Diseases Society of America (IDSA) has published guidelines for performing validation. These guidelines can be accessed at: http://www.idsociety.org/Topics_of_Interest/Antimicrobial_Resistance/Professionals/Antimicrobial_Susceptibility_Testing/. These IDSA guidelines recommend testing a minimum of 30 isolates including carbapenem susceptible, ESBL, and KPC-producing Enterobacteriaceae.

Reinforcing the above recommendation, the 21st Century Cures Act (Cures Act) enacted in 2016 requires the FDA to post information online about FDA's recognition, or withdrawal from recognition, in whole or in part, of breakpoints established by a standards development organization. This information should be referred to as the most up-to-date breakpoints for antibacterial and antifungal agents. With certain exceptions and additions, FDA recognizes the standard published in CLSI M100. With regard to CRE the FDA recognizes the current CLSI M100 breakpoints (first described in M100-S21) without exception.

References:

1. Clinical Laboratory Standards Institute (CLSI). 2018. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Eighth Informational Supplement M100- S28. CLSI Wayne, PA, USA.

SPECIFIC GUIDANCE: SUSCEPTIBILITY TESTING

Guidance for CRE screening in *E. coli*, *Enterobacter* spp and *Klebsiella* spp. will be described for the following susceptibility testing methods:

- Disk diffusion
- Gradient diffusion
- Automated systems (e.g., MicroScan®, VITEK®2, Phoenix™)

Note: Imipenem MICs for *Proteus* spp., *Providencia* spp., and *Morganella morganii* tend to be higher (that is, MICs in the I or R range) than meropenem or doripenem. These isolates may have resistance by mechanisms other than production of carbapenemases. For more information on screening among these organisms, view the FAQs in appendix A.

DISK DIFFUSION METHOD

The recommendations below are for laboratories using disk diffusion for routine susceptibility testing of carbapenems in Enterobacteriaceae. Disk diffusion is considered a reference method for susceptibility testing (reference M02) and therefore current (first described in M100-S21) CLSI breakpoints for carbapenems can be implemented immediately (See Table 2).

Test one or more of the carbapenems according to your protocol. Follow the recommendations of the manufacturer for performing the disk diffusion procedure.

- If an isolate tests R to ertapenem, meropenem, doripenem, or imipenem by current breakpoints (first described in M100-S21)

Then the organism should be considered a CRE.

GRADIENT DIFFUSION METHOD

This method consists of a predefined gradient of antibiotic concentrations on a plastic or paper strip which is used to determine the MIC of a particular antimicrobial agent. At the time of this writing, there are two commercially available strips: the E-Test® (BioMerieux) and the MTS (Liofilchem, Inc). The E-Test® has FDA-cleared strips for all four carbapenems; the MTS is only FDA-cleared for meropenem. The screening recommendations below are for laboratories using gradient strips for routine testing of carbapenems and for those that use gradient strips in addition to their automated systems to expand the dilution range to allow for application of the current breakpoints (first described in M100-S21).

Test one or more of the carbapenems according to your protocol. Follow the recommendations of the manufacturer for performing the gradient diffusion procedure.

- If an isolate tests R to ertapenem, meropenem, doripenem, or imipenem by current breakpoints (first described in M100-S21)

Then the organism should be considered a CRE.

AUTOMATED SYSTEMS

The screening recommendations below are for laboratories using automated systems MicroScan® WalkAway® (Beckman Coulter Diagnostics), VITEK® 2 (BioMérieux Diagnostics), or Phoenix™ (BD Diagnostics) for routine testing of carbapenems. All automated systems have alert rules for CRE based upon pre-defined conditions set up in the system software. These conditions are determined based on the card/panel type selected and the antimicrobials and MIC concentration range tested. Isolates exhibiting the pre-defined resistance patterns will be flagged as possible CREs. For more specific information about the pre-defined criteria for CRE flagging on your system, please contact your manufacturer representative.

The 2014 version of this guidance document included CRE testing and reporting strategies for laboratories that had not adopted the current breakpoints (first described in M100-S21) for cephalosporins and carbapenems. Many laboratories had not adopted the current breakpoints largely due to a lack of FDA cleared panels/cards that included the lower breakpoints. There are now card/panel options for each manufacturer that contain MIC concentration ranges consistent with current (i.e., lower) CLSI and FDA breakpoints, and laboratories should migrate their testing platforms to these new panels/cards.

The guidance below describes the CRE criteria for several common automated AST and is based on CLSI recommendations described in Table 2A of the M100-S28 document (2).

- •If an isolate tests R to ertapenem, meropenem, doripenem, or imipenem by current breakpoints (first described in M100-S21)

Then the organism should be considered a CRE

References

1. Clinical Laboratory Standards Institute (CLSI). 2018. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Eighth Informational Supplement M100- S28. CLSI Wayne, PA, USA.

2. Clinical Laboratory Standards Institute (CLSI). 2018. Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard-Thirteenth Edition. CLSI document M02-A13. CLSI Wayne, PA, USA.

SPECIFIC GUIDANCE: TESTING FOR CARBAPENEMASES

SCREENING AND CONFIRMATORY TESTING FOR CARBAPENEMASE PRODUCTION

Flagging suspected organisms: Isolates that test resistant to one or more of the carbapenems (doripenem, imipenem, meropenem or ertapenem) by any susceptibility testing method should be flagged as CRE.

While confirmatory testing for carbapenemase production is not necessary for clinical intervention we recommend confirmatory testing for carbapenemase production whenever possible for all Enterobacteraciae identified as resistant to any carbapenem for infection prevention, public health response and surveillance purposes. Additionally, laboratories that have not adopted current breakpoints (first described in M100-S21) for carbapenems described in Tables 2 and 3 should perform additional testing for evaluation of carbapenemase production in Enterobacteriaceae with MICs of 2-4 µg/mL for meropenem, imipenem, or doripenem and an MIC of 2 µg/mL for ertapenem.

PHENOTYPIC METHODS FOR THE DETECTION OF CARBAPENEMASE PRODUCTION

Currently there are several phenotypic and molecular tests that can be used for the identification of CP-CRE: the CARBA NP test, and the modified Carbapenem Inactivation Method (mCIM) and the Modified Hodge Test (MHT). Table 4 summarizes the characteristics of these phenotypic methods.

Method limitations: The class of carbapenemase cannot be determined by the results of phenotypic testing.

Interpretation of carbapenem susceptibility results: Laboratories that have not implemented the current CLSI carbapenem breakpoints should report all carbapenems as resistant regardless of the MIC or zone of inhibition when isolates are identified as CarbaNP or mCIM positive. Laboratories using current CLSI carbapenem breakpoints should not change the interpretation of carbapenem susceptibility results for CARBA NP or mCIM positive results.

For the purpose of this document, the mCIM is the phenotypic test recommended for assessment of carbapenemase production in Enterobacteriaceae. The MHT can be used for detecting KPC in *Klebsiella* spp. until the laboratory has validated and implemented the mCIM test

Table 4. Comparison of Carbapenemase Phenotypic Tests

	MHT*	CARBA NP	mCIM
Ease of use	Easy to perform and uses readily available reagents	Needs special reagents, cumbersome to prepare	Relatively easy to perform and uses readily available reagents
Time to result	24 hours (requires overnight incubation)	10 minutes to 2 hours	24 hours (requires overnight incubation)
Interpretation	Subjective	Subjective	Subjective but less problematic
Advantages	Simple to set up Sensitive for detecting KPC in *Klebsiella* spp.	Rapid results Sensitive and specific for detection of carbapenemases in Enterobacteriaceae and *P. aeruginosa*	Sensitive and specific for detection of all types of carbapenemases in Enterobacteriaceae
Limitations	Poor sensitivity and specificity. False positive in *Enterobacter* possessing Amp C and porin mutations False negatives with NDM-1 carbapenemases No longer a recommended phenotypic method for detecting carbapenemases	Poor sensitivity for detection of OXA- 48 carbapenemases Poor sensitivity and specificity for carbapenemases in *Acinetobacter*	Poor sensitivity and specificity for carbapenemases in *Acinetobacter*

Adapted from the CLSI M100-28. The procedures for performing the MHT and the mCIM test are described below. The CARBA NP test procedure is described in the CLSI M100-28 document, published in January 2018.

* The Modified Hodge Test (MHT) is no longer recommended for confirmation of carbapenemase production. New methods recommended by CLSI are described in this section.

Modified Carbapenem Inactivation Method (mCIM)

Guidelines below are reprinted with permission by the Clinical and Laboratory Standards Institute from: CLSI. Performance Standards for Antimicrobial Susceptibility Testing 28th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

Principle

Carbapenemase production is detected by the mCIM test when the test isolate produces the enzyme, which allows growth of a carbapenem susceptible strain (*E.coli* ATCC 25922), around the carbapenem disk.

Materials Required:

- TSB in 2 mL aliquots
- Meropenem disks (10 µg)
- 1-µL and 10-µL inoculation loops
- Mueller Hinton, TSB or normal saline (3-5 mL aliquots)
- Mueller Hinton agar plates (100 mm or 150 mm)
- Meropenem-susceptible indicator strain – *E. coli* ATCC 25922

Test Procedure:

1. For each isolate to be tested, emulsify a **1-µL loopful** of bacteria from an overnight blood agar plate in 2 mL TSB.
2. Vortex for 10-15 seconds.
3. Add a 10 µg meropenem disk to each tube using sterile forceps or a single disk dispenser. Ensure the entire disk is immersed in the suspension.
4. Incubate at 35°C ± 2°C in ambient air for 4 hours ± 15 minutes.
5. Just before or immediately following completion of the TSB-meropenem disk suspension incubation, prepare a 0.5 McFarland suspension (using the direct colony suspension method) of *E. coli* ATCC 25922 in nutrient broth or saline.
6. Inoculate a Mueller Hinton agar (MHA) plate with *E. coli* ATCC 25922 as for the routine disk diffusion procedure making sure the inoculum suspension preparation and MHA plate inoculation steps are each completed within 15 minutes. Allow the plates to dry for 3-10 minutes before adding the meropenem disks.
7. Remove the meropenem disk from each TSB-meropenem disk suspension using a **10-µL** loop by placing the flat side of the loop against the flat edge of the disk and using surface tension to pull the disk out of the liquid. Carefully drag and press the loop along the inside edge of the tube to expel excess liquid from the disk. Continue using the loop to remove the disk from the tube and then place it on the MHA plate previously inoculated with the meropenem-susceptible *E. coli* ATCC 25922 indicator strain. Disk capacity: 4 disks on a 100 mm MHA plate; 8 disks on a 150 mm MHA plate.
8. Invert and incubate the MHA plates at 35°C ± 2°C in ambient air for 18-24 hours.
9. Following incubation, measure the zones of inhibition as for the routine disk diffusion method.

Test Interpretation (See Figures 1 and 2)**:**

1. **Carbapenemase positive**: Zone 6-15 mm or presence of colonies within a 16-18 mm zone. If the test isolate produces carbapenemase, the meropenem in the disk will be hydrolyzed and there will be no inhibition or limited growth inhibition of the meropenem-susceptible *E. coli* ATCC 25922.

2. **Carbapenemase negative:** Zone ≥ 19 mm (clear zone). If the test isolate does not produce carbapenemase, the meropenem in the disk will not be hydrolyzed and will inhibit growth of the meropenem-susceptible *E. coli* ATCC 25922.

3. **Indeterminate:** Zone 16-18 mm or zone diameter of ≥ 19 mm and the presence of pinpoint colonies within the zone. The presence or absence of a carbapenemase cannot be confirmed.

Troubleshooting:

1. For indeterminate results:
   - Check test isolate and *E. coli* ATCC 25922 indicator strain for purity.
   - Check meropenem disk integrity by confirming acceptable results were obtained when disks were subjected to routine disk diffusion test QC.
   - Repeat the mCIM for test isolate and QC strains.
   - If the repeat test is indeterminate, consider performing a test for carbapenemase genes.
2. Some isolates may demonstrate colonies within the meropenem zone. If the zone measurement is ≤ 18 mm, this should be considered a carbapenemase positive result. However, if the zone is ≥ 19 mm, the result is indeterminant.
3. CLSI has currently standardized mCIM for Enterobacteriaceae and P. aeruginosa.

Reporting:

- **Positive:** Report "Carbapenemase detected."
- **Negative:** Report "Carbapenemase not detected."
- **Indeterminate:** Report "Testing inconclusive for the presence of carbapenemase. Call laboratory to discuss."

Quality Control:

Test positive and negative QC strains each day of testing:

- *Klebsiella pneumoniae* ATCC BAA-1705 – Carbapenemase positive
- *Klebsiella pneumoniae* ATCC BAA-1706 – Carbapenemase negative

In addition, perform QC of meropenem disks and test media daily or weekly following the routine disk diffusion QC procedure and handle disks as described in CLSI document M02. Alternatively, perform QC of meropenem disks with each run by removing a disk from the cartridge of disks used for the run and placing it on the MHA plate inoculated with *E. coli* ATCC 25922; incubate as above.

Validation of mCIM test:
A limited validation of at least 30 isolates (15 carbapenemase positive, 15 carbapenemase negative) needs to be performed to ensure the accurate performance (accuracy and precision) of the test in your laboratory. Characterized isolates known to be carbapenemase-producers from your institution should be used, if possible. (Reference: CLSI AST News Update. Volume 2, Issue 1, June 2017)

If an isolate is mCIM-positive, then the organism should be considered a CP-CRE. It is not necessary to test an isolate by mCIM when all carbapenem agents that are reported by a laboratory produce resistant results. However, mCIM may be performed for infection prevention or epidemiologic investigation to confirm carbapenemase production.

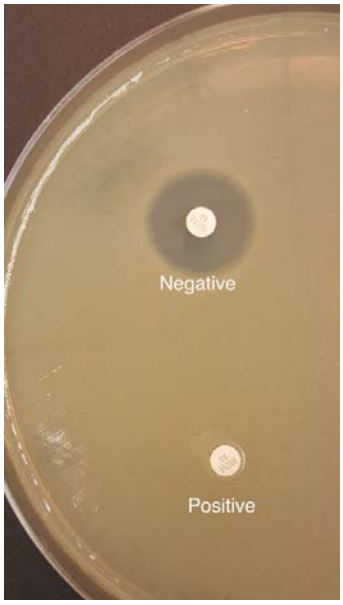


Figure 1. Modified Carbapenem Inactivation Method (mCIM) showing a negative (top) and a positive result (bottom).
mCIM positive has growth of the meropenem-susceptible *E. coli* ATCC 25922 up to the disk.
mCIM negative has an area of inhibition around the meropenem disk indicating inhibition of growth of the meropenem-susceptible *E. coli* ATCC 25922

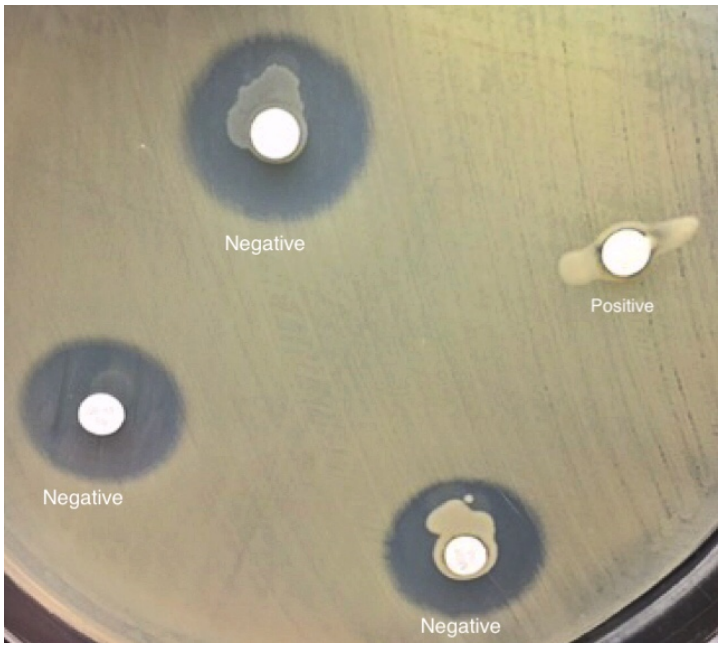


Figure 2. Modified Carbapenem Inactivation Method (mCIM) showing growth around the meropenem disk from carryover of the test organisms in the TSB and should be ignored when measuring the inhibition zone.

Modified Hodge Test (MHT) Method

MHT is no longer recommended for confirmation of carbapenemase production. However, based on data from the latest capacity survey showed the majority of NC facilities performing any further characterization still relied on MHT. Therefore, guidance on the practice (for use only in identifying detecting KPC in Klebsiella spp.) is provided. However, we encourage facilities to transition to other testing practices. New methods recommended by CLSI are described in this section.

NOTE: A negative result does not rule out the presence of carbapenemanses as the MHT is not sensitive or specific for detecting carbapenemases other than KPC in *Klebsiella* spp.. Some isolates show a slight indentation but do not produce carbapenemase.

Principle:
Carbapenemase production is detected by the MHT when the test isolate produces the enzyme, which allows growth of a carbapenem susceptible strain (*E.coli* ATCC 25922), towards a carbapenem disk. The result is a characteristic cloverleaf-like indentation (Figure 3).

Materials Required:

1. Sterile cotton tipped swabs
2. 1 mL sterile pipette or pipette and tips
3. 3 mL Mueller Hinton broth (MHB) or saline
4. Turbidity meter

5. Sterile loop or a sterile swab
6. Mueller Hinton agar plate
7. 10 µg meropenem disk
8. *E. coli* ATCC 25922, *K. pneumoniae* ATCC BAA-1705 and *K. pneumoniae* ATCC BAA-1706: 18-24hr subculture
9. Test organisms: 18-24hr subculture

Quality Control:
Perform quality control with each run.

- *Klebsiella pneumoniae* ATCC BAA-1705 – Carbapenemase positive
- *Klebsiella pneumoniae* ATCC BAA-1706 – Carbapenemase negative

Procedure:
Day 1 Testing

1. Prepare a 0.5 McFarland dilution of the *E.coli* ATCC 25922 in broth or saline or MicroScan inoculum water
2. Dilute by adding 0.3 mL of the 0.5 McFarland to a new 3mL of MHB or saline or water
3. Streak a lawn using the diluted inoculum of *E.coli* ATCC 25922 to a Mueller Hinton agar plate
4. Allow to dry 3-5 minutes
5. Place a 10 µg meropenem or ertapenem susceptibility disk in the center.
6. Using a 10 µl loop or sterile swab, pick up a visible inoculum from 3-5 colonies of the test organism
7. In a straight line, streak test organism from the edge of the disk to the edge of the plate. Up to four organisms can be tested on the same plate.
8. Incubate overnight at 35°C in ambient air for 16-24 hours

<u>Day 2 Reading and interpretation of results</u>

After 16-24 hours of incubation, examine the plate for a clover leaf type indentation at the intersection of the test organism and the *E. coli* 25922 within the zone of inhibition of the carbapenem susceptibility disk.

Interpretation of test results:

1. **Carbapenemase positive:** Presence of clover-leaf like indentation of the *E.coli* 25922 growing along the test organism growth streak within the disk diffusion zone.

2. **Carbapenemase negative:** No growth of the *E.coli* 25922 along the test organism growth streak within the disk diffusion zone.

Reporting:

- **Positive:** "Carbapenemase detected."
- **Negative:** "Carbapenemase not detected."

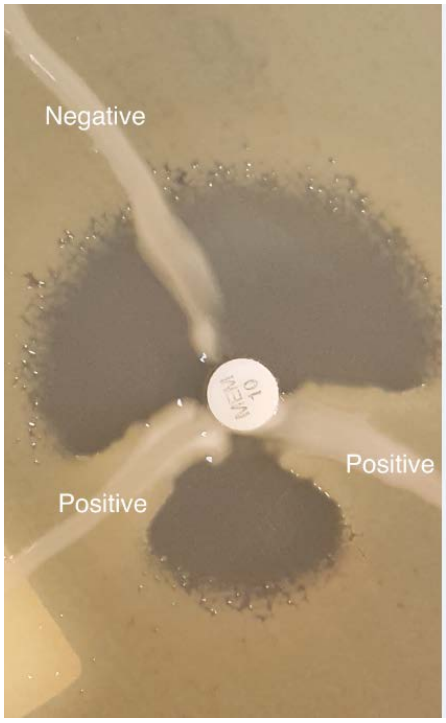


Figure 3. Modified Hodge Test (MHT) test showing two positive and one negative results
MHT Positive test has a clover leaf-like indentation of the *E.coli* 25922 growing along the test organism growth streak within the disk diffusion zone.
MHT Negative test has no growth of the *E.coli* 25922 along the test organism growth streak within the disk diffusion zone.

Additional phenotypic methods

There are some additional disk diffusion-based phenotypic methods that can be used to investigate the presence of carbapenamases (add reference). One commercial test recently cleared by FDA for detecting carbapenemases is the Rapidec Carba NP from BioMerieux.

MOLECULAR METHODS FOR DETECTION OF CARBAPENEMASE GENES

Laboratory-developed tests as well as commercially available molecular tests are available. Molecular methods are able to determine the type of carbapenamase in addition to determining the presence or absence of the gene encoding the carbapenemase. Some of the current commercially available assays can be used to detect one or more carbapenemases directly from blood cultures (FilmArray BCID panel from BioMerieux, Verigene Gram Negative Blood Culture Test from Luminex) or from an isolate (Xpert Carba R from Cepheid).

Some of the limitations of molecular tests are that special equipment or reagents may be needed and the cost is higher than the manual phenotypic tests described above. They are specific for the targeted genes and therefore a false negative result can be obtained if the carbapenemase gene present is not targeted.

ANTIMICROBIAL STEWARDSHIP AND THE LABORATORY

The Joint Commission issued a new standard for antimicrobial stewardship programs in hospitals, critical access hospitals, and nursing care centers in 2016 (1). The standard describes activities of the stewardship program that are aimed at antibiotic prescribers and consequently the membership of the antimicrobial stewardship team is expected, "if available in the setting," to consist of an infectious disease physician, infection preventionist(s), pharmacist(s), and a practitioner. Notably absent from the list of team members is a doctoral level clinical microbiologist or key member of the microbiology team, yet the contribution of a clinical microbiologist can significantly impact the efficacy of stewardship programs. The CDC supports participation of laboratorians as core members of the stewardship team (2).

While the main function of a clinical microbiology laboratory is to provide appropriate tests to aid in diagnosis and therapeutic management of a patient, this activity must be governed by judicious allocation of resources and evidence based approaches. The clinical microbiologist's job is not to strictly provide results from as many tests as possible but to provide rational use of tests to help the healthcare provider. In a similar manner antibiotic stewardship programs rely on the clinical microbiology laboratory to provide the information needed for effective stewardship activities. The stewardship contributions of the laboratory can be divided into three categories: (1) pre-analytical, (2) analytical, and (3) post-analytical. Examples of these contributions with a focus on those applicable to CRE are:

1. Pre-analytical (interventions that apply to the collection of a sample or the determination of appropriateness for analysis):
    a. Guidance on selection of tests (i.e. which specimens should have special resistance testing performed such as for carbapenemase production)
    b. Guidance on sample appropriateness for CRE/MDRO screening (i.e. in patients with chronic wounds)
2. Analytical (interventions that apply to the way a test or culture is performed)
    a. Implementation of the most current effective testing modalities (i.e. making sure current CLSI breakpoints are used, implementing rapid resistance testing and isolate identification technology when possible)
3. Post-analytical (interventions that apply to the interpretation of test results and how/when certain results are shared with providers)
    a. Rapid and effective result reporting to mitigate development of resistance, risk of *Clostridium difficile* infection and spread of MDROs like CRE via rapid implementation of control measures such as contact precautions.
    b. Assistance with interpretation of results (i.e. susceptibility results)
    c. Selective susceptibility reporting
        i. Do not report susceptibilities for drugs that are inherently ineffective (i.e. reporting Trimethoprim sulfamethoxazole (SXT) susceptibilities for *Enterococcus* species)
        ii. Cascade susceptibility reporting (i.e., not reporting carbapenem susceptibilities

for an organism that is ceftriaxone susceptible)

d. Sharing observations regarding microbiologic trends within a facility with the stewardship team (i.e., an increase in CRE isolates within a specific timeframe).
e. Providing or contributing to a facility antibiogram

Other examples of how the microbiologist contributes to antimicrobial stewardship are found in Sautter and Halstead (3) and Barlam, et al. (4).

With expertise in testing methodologies and new test implementation, the clinical microbiologist is well positioned to work closely with the stewardship team. The clinical microbiologist plays an important role in antimicrobial stewardship and can contribute to the antibiotic stewardship team as detailed above. Clinical microbiology should be represented on all committees discussing antibiotic resistance and control so the clinical microbiologist can best elucidate how he/she can help.

References:

1. The Joint Commission. 2016. Approved: new antimicrobial stewardship standard. *Joint Commission Perspectives* 36:1-8.
2. Centers for Disease Control and Prevention. 2017, posting date. Core elements of hospital antibiotic stewardship programs. Centers for Disease Control and Prevention. [Online.]. https://www.cdc.gov/antibiotic-use/stewardship-report/pdf/stewardship-report.pdf
3. Sautter RL, Halstead DC. 2018. Need of the hour: addressing the challenges of multi-drug-resistant health care-associated infections and the role of the laboratory in lowering infection rates. *Clin.Microbiol.Newsletter* 40:11-16.
4. Barlam TF, Cosgrove SE, Abbo LM, MacDougall C, Schuetz AN, Septimus EJ, Srinivasan A, Dellit TH, Falck-Ytter YT, Fishman NO, Hamilton CW, Jenkins TC, Lipsett PA, Malani PN, May LS, Moran GJ, Neuhauser MM, Newland JG, Ohl CA, Samore MH, Seo SK, Trivedi KK. 2016. Implementing an antibiotic stewardship program: guidelines by the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 62:e51-77. https://www.ncbi.nlm.nih.gov/pubmed/27080992

APPENDICES

A. FREQUENTLY ASKED QUESTIONS

Testing

1. Why did CDC change the CRE definition in 2015?

The previous CDC CRE definition (non-susceptible to imipenem, meropenem, or doripenem, AND resistant to all third generation cephalosporins tested) missed some CP-CRE. The latest case definition (resistant to imipenem, meropenem, doripenem, or ertapenem OR documentation that the isolate possesses a carbapenemase) was implemented in January 2015. This new definition is more inclusive , increasing detection of CP-CRE.

2. Why is the Modified Hodge Test no longer recommended for CRE detection?

The Modified Hodge Test may produce inaccurate results in some situations, such as producing false positive results among *Enterobacter* spp. and *Citrobacter* spp. due to production of the AmpC beta-lactamase. False positive results may also be observed with organisms carrying certain extended-spectrum beta-lactamases. Additionally, isolates producing the New Delhi metallo- β-lactamase (NDM) carbapenemase may go undetected by Modified Hodge Test. Modified Hodge Testing is also subject to misinterpretation. Other phenotypic tests for carbapenemase production such as the mCIM should be used if available.

3. Why did CLSI change carbapenem breakpoints to lower MICs?

CLSI changed carbapenem breakpoints in light of pharmacokinetic data that supported lower MICs. Lowering the MICs allows for the detection of low level producers of carbapenemases. It is important to note that low level resistance to one carbapenem does not preclude use of another carbapenem for treatment if that carbapenem tests as susceptible.

4. I am using disk diffusion; how can I implement the current breakpoints (first described in M100-S21) for carbapenems?

If the laboratory is using disk diffusion routinely and the staff is competent in performing and reading the results, only a limited verification study (using QC strains and clinical isolates) is needed before the lab can implement the current breakpoints (first described in M100-S21) for disk diffusion.
If the laboratory does not perform disk diffusion routinely, a full verification is recommended testing a minimum of 30 isolates. In addition, the lab staff should have completed a competency assessment for performing the disk diffusion test.

5. I am using a commercial antimicrobial susceptibility system; how can I implement the new breakpoints (first described in M100-S21) for carbapenems?

A laboratory can use the current breakpoints by: changing the current panel to a panel which is cleared for the current breakpoints OR performing validation of the current breakpoints using their current panels. See section "carbapenem breakpoints" in this document

6. Do I need to perform validation before implementing the mCIM test?

Yes, see the mCIM procedure in the section "Phenotypic Methods for the Detection of Carbapenemase Production" in this document.

7. What do I need to do if the isolate is resistant to ertapenem only?

This isolate still meets the CDC case definition for CRE. This isolate can undergo additional testing for carbapenemase production for infection prevention and public health purposes. If additional testing is not available, the isolate can be sent to the State Laboratory of Public Health for mechanism testing (see further information in the Reporting section below).

Reporting

1. What should be reported to DPH?

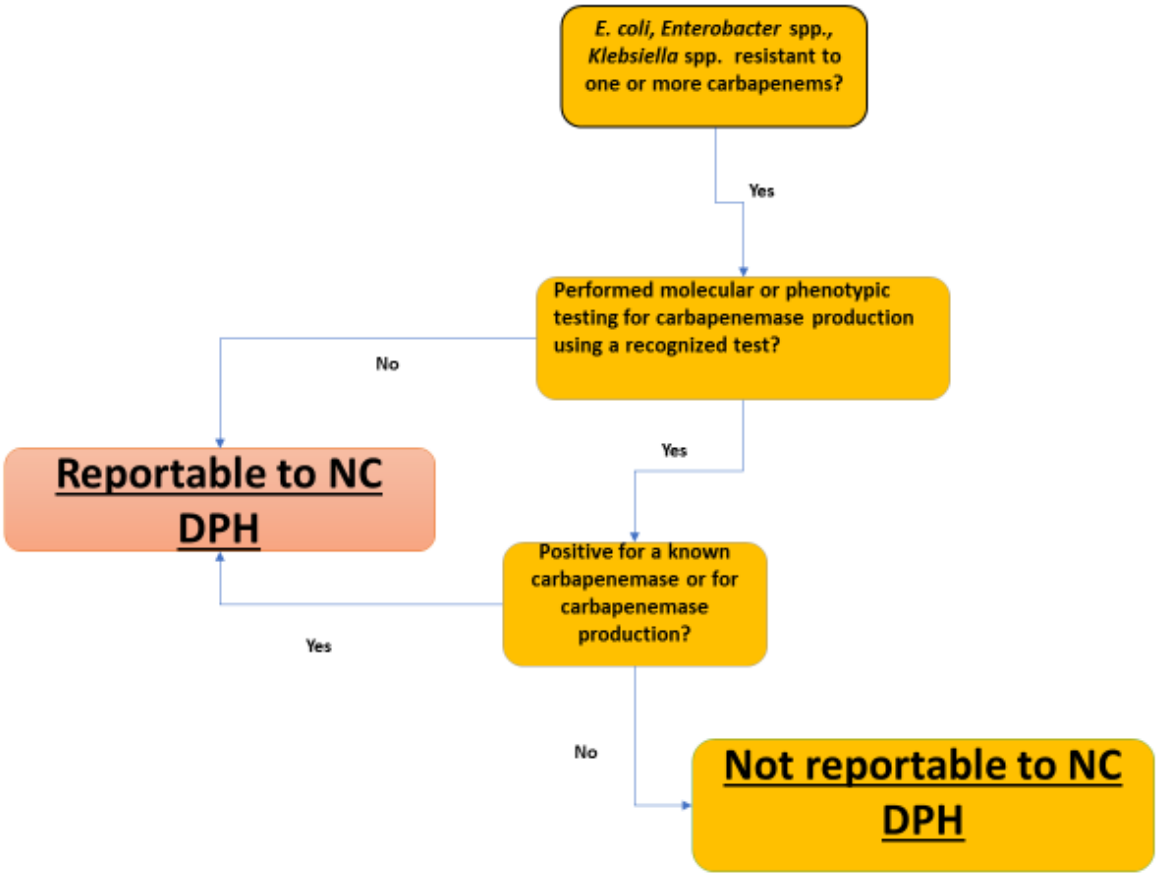
For the purposes of reporting, Carbapenem-Resistant Enterobacteriaceae (CRE) are defined as:

(1) *Enterobacter* spp., *E. coli* or *Klebsiella* spp. positive for a known carbapenemase resistance mechanism or positive on a phenotypic test for carbapenemase production; or
(2) *Enterobacter* spp., *E. coli* or *Klebsiella* spp. resistant to any carbapenem in the absence of carbapenemase resistance mechanism testing or phenotypic testing for carbapenemase production under the current CLSI breakpoints first described in M100-S21.

Additionally, identification of a CRE producing a carbapenemase other than KPC, even among Enterobacteriaceae other than *E. coli, Enterobacter* spp., *Klebsiella* spp. represents a containment opportunity. While not described in reporting requirement, it is requested that these are reported to DPH.

DPH is also available to assist in cluster or outbreak response, regardless of the mechanism of resistance.

CRE should be reported regardless of whether it was collected for clinical or screening purposes.



2. When should I report if I am still using the old CLSI breakpoints?

We do not directly address the old CRE CLSI breakpoints in these guidelines as all labs should be using the M100-S21 breakpoints as recommended by the FDA and discussed in the breakpoints for carbapenems section. If you are in the process of transitioning to M100-S21 please report any identified CRE and provide MIC qualitative and quantitative values.

3. How should results be reported to DPH?

Report identification of CRE from a clinical specimen associated with either infection or colonization, including susceptibility results and all phenotypic or molecular test results. Results should be submitted via electronic laboratory reporting (ELR); when ELR is not available the result should be faxed to 919-715-4760.

4. When should carbapenem resistant organisms be sent to the State Laboratory of Public Health?

Enterobacter spp., *E. coli* or *Klebsiella* spp. resistant to any carbapenem in the absence of carbapenemase resistance mechanism testing should be sent to the State Laboratory of Public Health. Isolates should be sent regardless of whether they were collected for clinical or surveillance purposes.

While not reportable, identification of CRE producing a carbapenemase other than KPC, among Enterobacteriaceae other than *E. coli, Enterobacter* spp.*, Klebsiella* spp. may be requested for additional testing.

5. If a laboratory determines the resistance gene for an isolate, does the isolate need to be sent to the State Laboratory of Public Health?

If the facility is appropriately using recognized testing methods, then isolates testing positive for a known carbapenemase resistance mechanism do not need to be routinely sent to the State Laboratory of Public Health. However, if the facility identifies a novel or uncommon mechanism of resistance or if the isolate is identified as part of a possible cluster or outbreak the state laboratory may request the isolate for additional and/or confirmatory testing.

Additionally, if your facility identifies an isolate with discordant phenotypic and molecular results for carbapenemase production (positive via phenotypic test but negative via PCR or vice versa), please arrange to have these isolates sent for additional testing.

6. How should isolates be sent to the State Laboratory of Public Health?

Fill out form DHHS #3390 (https://slph.ncpublichealth.com/Forms/3390-EntericBacteriology-20170808.pdf) with the following required information:

- First and last name of patient
- Address of patient
- Medical Record Number of patient
- Name and address of submitter
- Collection date
- For "Specimen Type" select "Isolated organism" and provide the identification of the organism
- Source
- For "Test Ordered" select other and write "CRE surveillance"

Use the Microbiology Reference mailer or equivalent to ship pure cultures. Please contact Shermalyn Greene (Shermalyn.Greene@dhhs.nc.gov) if shipping materials are needed or if transport assistance is needed. Submitted isolates should be on agar slants. If available, provide any test results.

To submit specimens:

- Write patient's name and other identifier on the slant Place completed Enteric Bacteriology DHHS form #3390 (one form for each specimen) in outer container to avoid contamination in case of breakage or leakage.
- Use double-walled or equivalent shipping containers that meet safety requirements.
- Multiple tubes should be wrapped individually in absorbent cushioning material and securely packaged in a leak-proof container. Include "Attention: Shermalyn Greene, CRE" on the shipping containing.
- When shipping by U.S. mail (slant containers), use first-class postage. Please contact Shermalyn Greene if FedEX is needed. Be sure to place return address on outside of container, regardless

of shipping method.

Ship to:
For USPS and State Courier Deliveries
North Carolina State Laboratory of Public Health
4312 District Drive
1918 Mail Service Center
ATTN: Shermalyn R. Greene CRE Surveillance
Raleigh, NC 27699-1918

For FedEX Deliveries
Shermalyn R. Greene
North Carolina State Laboratory of Public Health
4312 District Drive
Raleigh, NC 27607

7. How should results of the carbapenems be reported if phenotypic testing is positive?

CLSI recommends laboratories that have not implemented the current CLSI carbapenem breakpoints report all carbapenems as resistant regardless of the MIC or area of inhibition when isolates are identified as CarbaNP or mCIM positive.

As per CLSI recommendations laboratories using current CLSI carbapenem breakpoints should not change the interpretation of carbapenem susceptibility results for CARBA NP or mCIM positive results. Laboratories should consult with their infectious disease or antimicrobial stewardship teams to decide on the reporting strategy that best fits their particular clinical needs.

A phenotypic test classifies the CRE as carbapenemase producing but does not specify the mechanism of resistance. When feasible, these isolates may be sent to the State Laboratory of Public Health for molecular testing.

8. I am using the current breakpoints (first described in M100-S21). Do I need to perform the mCIM test before sending the isolate to the State Laboratory of Public Health?

This is a decision that can be made based on your current laboratory capacity. If no additional testing is done, report and send all CRE to the State Laboratory of Public Health. If mCIM is performed, submit only those that test positive for carbapenemase production.

9. What results should be reported (e.g., CRE detected, CP-CRE detected, specific R gene, etc.) to providers?

All results should be reported including susceptibility, phenotypic and molecular test results. Your facility may need to provide education to ensure results are appropriately interpreted. Decisions regarding facility specific practices to assist with interpretation of susceptibility results for CRE isolates should be interdisciplinary, including a team of stewardship experts (infectious disease physician, infection preventionist(s), pharmacist(s), practitioner(s), clinical microbiologist(s), etc.). One suggested practice is the addition of a comment or footnote to these results alerting providers that the organism is a CRE and carbapenems may not be effective. With the exception from question 5 above (phenotypic positive tests on CRE isolates tested at laboratories using current breakpoints, first described in M100-S21), changing the interpretation of carbapenem susceptibility results is not recommended by CLSI. However, if your institution's stewardship team chooses to edit the interpretations of the Carbapenem susceptibility results to R for CRE, the original zone of inhibition readings and/or unedited MIC results should be saved as this is valuable information to future providers as well as public health.

Surveillance

1. Why conduct public health surveillance?

Surveillance of HAIs can provide insight into the specific prevalence of infections in North Carolina and provide the data needed to develop prevention and treatment strategies across the state. Early detection and aggressive implementation of infection prevention and control strategies are necessary to prevent further spread of CRE. These strategies require an understanding of the prevalence or incidence of these conditions. Surveillance allows for prompt notification of public health authorities when cases of CRE are detected to contain these organisms. Required reporting and subsequent analysis of surveillance data will inform the development, implementation and evaluation of prevention and public health control measures used to contain CRE.

2. How does a clinical lab contribute to surveillance?

Facilities can contribute by ensuring the use of appropriate methods for identification of CRE and performing periodic reviews of laboratory data to quantify incidence of CRE and detect changes in overall trends. When a facility reports CRE to NC DPH and/or submits CRE isolates for mechanism testing they contribute to the complete picture of what is happening in regard to resistance in North Carolina. These data are used to inform public health action.

Other

1. Who can I contact if I have additional questions?

The coauthors of this publication are available for discussion and clarification as needed.
You can email questions to the NC DPH Surveillance for Healthcare Associated and Resistant Pathogens Patient Safety Program SHARPPS team at NCHAI@dhhs.nc.gov

ABBREVIATIONS

AST	Antimicrobial susceptibility test
CAP	College of American Pathologists
Carba NP	Carba Nordmann- Poirel
CDC	Centers for Disease Control and Prevention
CIM	Carbapenem Inactivation Method
CLIA	Clinical Laboratory Improvement Amendments
CSLI	Clinical Laboratory Standards Institute
CRE	Carbapenem-Resistant Enterobacteriaceae
CP-CRE	Carbapenemase-Producing Carbapenem-Resistant Enterobacteriaceae
CTX-M	Type of Carbapenemase (Class A β-lactamase)
ESBL	Extended Spectrum Beta-Lactamase
FDA	Food and Drug Administration
GES	Type of Carbapenemase (Class A β-lactamase)
I	Intermediate
IDSA	Infectious Diseases Society of America
IMP	Imipenemase
KPC	*Klebsiella pneumoniae* carbapenemase
mCIM	Modified Carbapenem Inactivation Method
µg	Microgram
MHT	Modified Hodge Test
MIC	Minimum Inhibitory Concentration
ml	Milliliter
mm	Millimeters
NC DPH	North Carolina Division of Public Health
NDM	New Delhi Metallo-β-Lactamase
NC SLPH	North Carolina State Laboratory of Public Health
OXA	Oxacillinase (Class D β-lactamase)
PCR	Polymerase Chain Reaction
R	Resistant
SME	*Serratia marcescens* enzyme (Class A β-lactamase)
spp.	Species
VIM	Verona Integron-mediated Metallo-β-Lactamase

RESOURCES

1. Management of Multidrug Resistant Organisms in Healthcare Settings, 2006 https://www.cdc.gov/hicpac/mdro/mdro_toc.html
2. Interim Guidance for a Public Health Response to Contain Novel or Targeted Multidrug-resistant Organisms (MDROs) https://www.cdc.gov/hai/outbreaks/docs/Health-Response-Contain-MDRO.pdf
3. Facility Guidance for Control of Carbapenem-Resistant Enterobacteriaceae (CRE) https://www.cdc.gov/hai/pdfs/cre/CRE-guidance-508.pdf
4. Clinical Laboratory Standards Institute (CLSI). 2018. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Eighth Informational Supplement M100- S28. CLSI Wayne, PA, USA.
5. Clinical Laboratory Standards Institute (CLSI). 2018. Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard-Thirteenth Edition. CLSI document M02-A13. CLSI Wayne, PA, USA.