Burning Question - When Should Clinical Microbiology Laboratories Perform Carbapenemase Detection Tests?

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Carbapenem resistance is one of the most concerning forms of antimicrobial resistance, particularly when encountered in the *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*.[1,2] Organisms displaying overt resistance to carbapenems can be divided into two groups: 1) carbapenemase-producing organisms (CPO) that express carbapenemases, enzymes that hydrolyze the carbapenem β-lactam ring, and 2) non-carbapenemase-producing-carbapenem-resistant organisms (non-CP-CRO) that have reduced susceptibility to carbapenems due to expression of cephalosporinases (ESBL and AmpC) coupled with cell permeability defects.[1,2] Unlike non-CP-CRO, the genes associated with CPO are readily transferrable to many gram-negative species as these are often located on mobile genetic elements (eg, plasmids), increasing the potential for widescale spread.[1,2]

Carbapenemases belong to one of three classes based upon their amino acid sequence: Ambler class A, B, or D. Class A (eg, KPC) and D (eg, OXA-48-type) enzymes possess a serine-based hydrolytic mechanism, while class B carbapenemases (eg, NDM, IMP, VIM) are metallo-β-lactamases and require one or two zinc ions for catalytic activity.[3] KPC is endemic in the United States, Israel, South America and some countries in Europe and Asia, while OXA-48-type and NDM enzymes predominate in North Africa/Europe and Asia, respectively.[1,2] However, as a result of widespread international travel and exposure to medical care, the association between a specific resistance mechanism and a given region or country is not definite and may change.[1]

There are many phenotypic and genotypic carbapenemase detection tests (CDT) available for use in clinical laboratories **(see Table 1).**[1,2,4] Typically, phenotypic assays detect carbapenemase activity in bacterial isolates recovered in culture, while genotypic assays permit detection of carbapenemase genes directly in clinical specimens (eg, positive blood cultures or rectal swabs) or from organisms isolated in culture. Differentiation between non-CP-CRO and CPO is not recommended by the Clinical and Laboratory Standards Institute (CLSI) for routine patient care, except for those laboratories that have not yet implemented the current CLSI *Enterobacteriaceae* carbapenem breakpoints.[5] ***Therefore, why should Clinical Microbiologists consider CDT when confronted with the increasing challenges facing laboratories today? Reduced operational costs, lack of test charge reimbursement, and a shortage of individuals entering the profession, to list but a few.***

Table 1. Selection of Phenotypic and Genotypic CDT Currently Available (modified from [1,2,4]).

Test (Manufacturer)	Method	Specimen Type	Turnaround Time (time to results from setting up the assay)	Carbapenemase Gene(s) Detected	Regulatory Status
Phenotypic CDT					
Carba NP	Color indicator of imipenem hydrolysis	Isolates of *Enterobacteriaceae* or *P. aeruginosa*	Same day	Not applicable (N/A)	Commercial version United States Food and Drug Administration (FDA) cleared
mCIM	Growth of carbapenem susceptible indicator strain around meropenem disk incubated with a CPO test strain	Isolates of *Enterobacteriaceae* or *P. aeruginosa*	Next day	N/A	Laboratory Developed Test (LDT)
eCIM	Growth of carbapenem susceptible indicator strain around meropenem disk incubated with a CPO test strain in the presence and absence of EDTA	Isolates of *Enterobacteriaceae* (modification of mCIM that allows differentiation between serine- and metal-dependent carbapenemases)	Next day	N/A	LDT

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Table 1. Selection of Phenotypic and Genotypic CDT Currently Available (modified from [1,2,4]). (*Continued*)

Test (Manufacturer)	Method	Specimen Type	Turnaround Time (time to results from setting up the assay)	Carbapenemase Gene(s) Detected	Regulatory Status
Phenotypic CDT					
MALDI-TOF MS	Detection of carbapenem degradation products	Bacterial isolates	Same day	N/A	LDT
Genotypic CDT					
FilmArray® Blood culture identification panel (BioFire Diagnostics)	PCR	Positive blood culture broth with GNR	Same day	[a]bla_{KPC}	FDA cleared
Verigene® gram-negative blood culture test (Luminex Corporation)	Microarray	Positive blood culture broth with GNR	Same day	[b]bla_{IMP} bla_{KPC} bla_{NDM} bla_{OXA-48} bla_{VIM}	FDA cleared
GeneXpert® Carba-R (Cepheid)	PCR	Rectal swabs, isolates of *Enterobacteriaceae*, *P. aeruginosa*, *A. baumannii*	Same day	[c]bla_{IMP} bla_{KPC} bla_{NDM} bla_{OXA-48} bla_{VIM}	FDA cleared

Abbreviations: CPO, carbapenemase-producing organism; EDTA, ethylenediaminetetraacetic acid; GNR, gram-negative rods; LDT, laboratory developed test; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

[a]KPC resistance gene only reported when one of the following organisms is detected: *A. baumannii*, *Enterobacter cloacae* complex, *Escherichia coli*, *Klebsiella pneumoniae, Klebsiella oxytoca, Proteus* species, *P. aeruginosa, Serratia marcescens*.

[b]Carbapenemase resistance genes only reported when one of the following organisms is detected: *Acinetobacter* species, *Citrobacter* species, *Enterobacter* species, *E. coli, K. pneumoniae, K. oxytoca, P. aeruginosa, Proteus* species, *S. marcescens*.

[c]The Carba-R system does not perform organism identification, only molecular detection of carbapenemase genes.

First, as mentioned above, **if clinical laboratories have not implemented the current CLSI carbapenem breakpoints for *Enterobacteriaceae*** a CDT should be performed when isolates of *Enterobacteriaceae* exhibit a minimum inhibitory concentration (MIC) value of 2 µg/mL for ertapenem or 2-4 µg/mL for imipenem or meropenem.[5] However, laboratories should ardently strive to use current breakpoints for accurate identification of carbapenem resistance.

Second, controlling the spread of CPO, in particular carbapenemase-producing-carbapenem-resistant *Enterobacteriaceae* (CP-CRE), within institutions is critical. However, this is challenging because many CPO-infected patients are initially identified by routine antimicrobial susceptibility testing (AST), which may take up to five days to report. And CPO from diagnostic cultures represent the "tip of the iceberg" of patients harboring CPO. Therefore, some institutions have initiated surveillance for CPO (especially within their immunosuppressed patient populations) ranging from culture with selective and differential media with or without a CDT to molecular methods. Rapid CDT that screen for CPO colonization offer the opportunity to promptly implement infection control interventions resulting in reduced CPO transmission as demonstrated in practice.[2] Similarly, rapid CDT that detect and differentiate carbapenemases permit the identification of related cases during an outbreak.

Finally, infections with carbapenem-resistant organisms, especially bloodstream infections, remain difficult to treat and are associated with unacceptably high mortality rates.[1,2] Implementation of diagnostics that rapidly identify these organisms from positive blood cultures could improve patient outcomes by permitting earlier consultation with infectious diseases experts and prompt administration of effective empiric therapy. Indeed, consultation with infectious diseases specialists is linked to favorable outcomes for patients with *Staphylococcus aureus* bloodstream infections[6] and will likely benefit patients with invasive infections because of

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CPO. Importantly, newer antimicrobials active against CPO often depend on the carbapenemase type, and this knowledge prior to conventional AST results could support decisions about use of these agents for empiric therapy. For example, most KPC and some OXA-48-type producing isolates are susceptible to ceftazidime-avibactam, but this drug has no activity against isolates that produce metallo-β-lactamases.[1,2]

In summary, an institution's local CPO prevalence and patient population will largely dictate the economic and clinical benefit of introducing CDT. Clinical Microbiologists should actively engage infectious diseases and infection control and prevention specialists and their antimicrobial stewardship programs to determine the necessity, method and frequency of such testing. However for the reasons presented above, clinical microbiology laboratories are strongly encouraged to adopt, or have readily available access to, some form of CDT that permits accurate detection of CPO in their institutions. In addition, as mentioned above, all laboratories are strongly encouraged to use current CLSI recommended carbapenem breakpoints.

References

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