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REVIEW

Clinical and laboratory considerations for the rapid detection of carbapenem-resistant Enterobacteriaceae

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ABSTRACT

Carbapenem resistance among the Enterobacteriaceae has become a significant clinical and public health dilemma. Rapid administration of effective antimicrobials and implementation of supplemental infection control practices is required to both improve patient outcomes and limit the spread of these highly resistant organisms. However, carbapenem-resistant Enterobacteriaceae (CRE)-infected patients are predominantly identified by routine culture methods, which take days to perform. Rapid genomic and phenotypic methods are currently available to accelerate the identification of carbapenemase-producing CRE. Effective use of these technologies is reliant on close collaboration between clinical microbiology, infection prevention, antimicrobial stewardship and infectious diseases specialists. This review discusses the performance characteristics of these technologies to date, and describes strategies for their optimal implementation.

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Introduction

Carbapenem resistance among the Enterobacteriaceae has become a significant clinical and public health dilemma. Surveillance data demonstrate a steady increase in the burden of disease from CRE, in particular carbapenem-resistant *Klebsiella pneumoniae*, between 2000 and the present day.[1-3] In 2013, the US. Centers for Disease Control and Prevention estimated 9,300 patients a year were infected with carbapenem-resistant Enterobacteriaceae (CRE), with an associated 610 deaths.[4] Similar trends have been noted worldwide: in 2014, the World Health Organization (WHO) reported that up to 68% of *K. pneumoniae* were carbapenem resistant in Europe, 55% in South-East Asia, 54% in the Eastern Mediterranean region, 11% in the Western Pacific, 9% in the Americas and 3.5% in Africa.[5] However, the accuracy of these numbers is uncertain because the vast majority of countries that evaluate and report CRE prevalence are in the Americas and Europe. As such, resistance in some areas (e.g. Africa, Southeast Asia) may be underestimated.

β-lactam resistance among the Enterobacteriaceae is largely driven by the expression of enzymes that cleave the β-lactam ring. These β-lactamases are classified according to the Ambler system into 4 classes (A, B, C and D), based on their amino acid sequence. Class A includes the active-site serine β-lactamases, class B the metallo-β-lactamases, class C the AmpC β-lactamases and class D the oxacillinases. Each class harbors β-lactamases with varying activity against the carbapenem class of antibiotics. Frank carbapenem resistance is mechanistically divided into 2 broad groups: 1) non-carbapenemase producing (CP) CRE and 2) CP CRE. Non-CP isolates have reduced susceptibility to the carbapenems (in particular ertapenem) due to expression of an acquired class A or class C β-lactamase with weak carbapenem hydrolytic activity (e.g.,, extended spectrum β-lactamase (ESBL) or AmpC enzymes) that is coupled with a permeability defect (e.g., outer membrane porin mutation or loss). In particular, CTX-M ESBLs and CMY-2 AmpCs commonly contribute to reduced carbapenem susceptibility in non-CP CRE.[6] In contrast, CP-CRE are those isolates that express an acquired carbapenemase gene that specifically hydrolyzes the carbapenem β-lactam ring. Carbapenemases belong to the Ambler class A, B and C groups of β-lactamases, the genes for which typically are found on acquired plasmids, but may also be on other transmissible genetic elements inserted into the chromosome. In the US, the CRE epidemic is driven by the rapid expansion of CP-*K. pneumoniae* of sequence type 258, that express the class A KPC carbapenemase.[7,8] KPC is a particularly efficient enzyme and isolates with this resistance mechanism often display very high carbapenem minimum inhibitory concentrations

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(MICs). KPC is now endemic in the US, Israel, South America and some countries in Europe and Asia. In other areas of the world, class B New Delhi metallo-β-lactamase (Asia) or class D OXA-48-like carbapenemases (North Africa and Europe) predominate.[9,10]

One of the key challenges of controlling the spread of CRE is the fact that CRE-infected patients identified by routine clinical cultures (passive surveillance) represent only a fraction of the patients that harbor CRE. Gastrointestinal colonization is asymptomatic, and colonized patients are reservoirs for transmission. Once introduced into a health system by colonized patients, CP-CRE can rapidly disseminate within and across institutions.[11] Rapid identification of patients who are colonized with CP-CRE may allow implementation of infection control precautions to prevent transmission. Such testing has the potential to halt the spread of these highly resistant organisms at the local, regional and national level.

At present, nearly all patients infected or colonized with a CRE are identified by front-line susceptibility testing performed by the clinical laboratory on isolates recovered in culture. Such testing is associated with a 2–5 day delay between specimen collection and availability of results. Routine screening of CP-CRE colonization is not commonly performed. Furthermore, few clinical laboratories routinely perform supplemental testing to differentiate CP from non-CP isolates, as this is not required by either the Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for routine patient care, provided laboratories are using updated carbapenem breakpoints.[12,13] Given the critical relationships between identification of CP-CRE, timely initiation of effective antimicrobial therapy and infection control interventions, and patient outcomes, rapid tests for CP-CRE detection are clearly needed. This review will describe progress to date on the development of diagnostic tests to detect CP-CRE, their use and interpretation.

Diagnostic tests to detect CRE

Several commercial tests (either research use only (RUO) or FDA-cleared/CE marked) are available for more rapid detection of carbapenem resistance. These can be divided into 2 categories: molecular tests that detect the resistance mechanism (i.e. presence of a carbapenemase gene) and novel, phenotypic tests that detect the *in vitro* activity of carbapenemase enzymes (i.e., hydrolysis of carbapenems *in vitro*). Few-to-none of these tests can be performed directly on patient specimens, but rather require bacterial culture prior to testing.

Rapid nucleic acid-based tests

A plethora of reports describing nucleic acid amplification tests for the detection of carbapenemase genes have been presented in the literature over the past several years. Only a few of these have obtained CE mark or FDA clearance for clinical testing (Table 1), and the majority are either laboratory developed tests (LDT) or available for purchase as RUO. The advantages of nucleic acid-based molecular tests, as compared to conventional culture-based phenotypic tests, include: rapid turn around time; definitive identification of specific carbapenemase gene(s); and, in some cases, the ability to test directly from clinical specimens without the need for culture. The disadvantage of such tests is they only detect those enzymes queried by the primers and probes of the assay. This poses a problem for clinical diagnostic testing, as carbapenem resistance may be conferred by

Table 1. CE Mark and FDA-Cleared Tests for the Molecular Detection of CP-CRE.

Test (Manufacturer)	Specimen Type	Carbapenemase Gene(s) Detected	Regulatory Status	References
FilmArray® Blood Culture Identification Panel (BioFire)	Positive Blood Culture Broth	bla_{KPC}	US. FDA Cleared CE-IVD	19-22
Verigene® Gram-negative blood culture test (Nanosphere)	Positive Blood Culture Broth	bla_{KPC} bla_{IMP} bla_{VIM} bla_{NDM} bla_{OXA-48}	US. FDA Cleared CE-IVD	23-24
Unyvero® P55 (Curetis AG)	Respiratory secretions	bla_{KPC} bla_{IMP} bla_{VIM} bla_{NDM} bla_{OXA-48}	CE-IVD	25
GeneXpert Carba-R (Cepheid)	Rectal swabs	bla_{KPC} bla_{IMP} bla_{VIM} bla_{NDM} bla_{OXA-48}	US. FDA Cleared CE-IVD	26

Note. US. FDA, United States Food and Drug Administration; CE-IVD, Conformite Europeene *In Vitro* Diagnostic.

mechanisms other than carbapenemases (as described above), and false negative results may be observed if a novel carbapenemase variant emerges. For instance, several variants of the IMP carbapenemase exist, which are only variably detected by commercial PCR systems.[14] Similarly, in one of our laboratories, the emerging OXA-48-like variant, OXA-232, was not detected by a LDT designed to detect the OXA-48 gene, due to a single nucleotide mismatch in the primer region.[15] As such, these tests may be best suited to 'rule-in' as opposed to 'rule-out' infection with a CRE. From a clinical perspective, a positive result provides an early trigger to escalate therapy. Along these lines, a recent study evaluated the predictive values of 2 nucleic-acid based methods that detect carbapenemase genes, for determining carbapenem resistance. With the assumption of 5% carbapenem resistance in a given population, the tests both predicted susceptibility correctly in >95 % of cases, whereas prediction of resistance was only 41–50%, dependent on test method.[16]

A further complication of nucleic-acid based molecular testing is the occasional occurrence of isolates that test positive for a carbapenemase gene, but are susceptible *in vitro* to the carbapenems. For instance, isolates that test susceptible to ertapenem, meropenem and imipenem, but harbor the bla_{KPC} gene, have been described.[17] These isolates typically express only low levels of KPC and harbor intact OmpK35 and OmpK36 outer membrane porins.[17] Alternatively, such isolates may have lost the bla_{KPC} -harboring plasmid in the time interval between molecular testing and MIC testing.[17] The clinical significance of such findings is unclear, but both CLSI and EUCAST suggest reporting carbapenem susceptibility results as-tested using updated carbapenem breakpoints, rather than editing these to resistant, if a carbapenemase gene is detected.[13,18] More research is needed to understand the clinical significance of such discordance and how best to treat isolates that contains a "silent" carbapenemase gene, yet displays carbapenem susceptibility.[16]

Tests performed directly from blood cultures and clinical specimens

Two tests are currently available that detect carbapenemase genes in bacteria present in blood: the FilmArray® Blood Culture Identification Panel (BCID, BioFire Diagnostics LLC, Salt Lake City, UT) and the Verigene® Gram-negative blood culture test (Nanosphere, Northbrook, IL). Both tests require blood to be cultured on a standard laboratory automated blood culture system prior to testing. For most Gram negative bacteria, this step takes 8–24 hours from instrument loading to culture positivity.[19] This culture step is critical because nucleic acid amplification tests performed directly from blood have not yet achieved sensitivity equivalent to blood culture. In contrast, coupling nucleic acid amplification and/or detection tests with a pre-culture step allows biological amplification of the organisms and subsequent high sensitivity for the targeted resistance genes.

The FilmArray® BCID is a multiplex PCR that identifies 24 microorganisms commonly encountered in blood, including Gram-positive bacteria, Gram-negative bacteria, and *Candida* spp. Additionally, the test targets one carbapenemase gene, bla_{KPC}, along with the Gram-positive resistance markers, *mecA, vanA* and *vanB*. The US clinical trial was performed across 8 clinical sites and evaluated 1568 clinical blood cultures. Only 6 were positive for bla_{KPC}, which was confirmed by reference methods. An additional 33/33 KPC-producing organisms seeded into blood were detected by the system.[20] No false-positive bla_{KPC} results were observed.[20] Some investigators have applied this technology to specimens other than blood (i.e., normally sterile body fluids and respiratory secretions), performing the test directly from the specimen without the benefit of pre-culture. As might be expected, the sensitivity for the detection of bacteria in these specimens was lower than what is observed for blood cultures (i.e., 71–89% sensitivity of the BCID as compared to conventional cultures).[21] This study did not include any CRE, so it is unclear what the performance for the detection of resistance genes might be. The main limitation of the BCID is the inclusion of only bla_{KPC} as a target. While this carbapenemase gene is by far the most common across the US, organisms expressing other carbapenemases are encountered sporadically in this country, and are common in other areas of the world.[22] Nonetheless, knowledge of the presence of bla_{KPC} may aid in rapid escalation of therapy, potentially improving patient outcomes.

The Verigene® Gram-negative test is a microarray-based method that detects the 8 most common Gram-negative bacteria encountered in blood and the following carbapenemases: IMP, KPC, NDM, OXA and VIM, along with CTX-M ESBLs. Two large, multi-center studies have evaluated the analytical performance of this assay to date. The first was the US clinical trial, which evaluated a total of 1847 blood cultures positive for Gram-negative bacteria, including 337 simulated specimens, across 13 clinical centers. This study identified only 16 patients (<1 %) with blood cultures positive for a carbapenemase gene, including bla_{KPC} (n = 3), bla_{NDM} (n = 1) and bla_{OXA} (n = 12). The Verigene system accurately detected all but 3 bla_{OXA} (false negatives), which were determined to be the $bla_{OXA\text{-}23}$ variant not targeted by the assay. Additionally, among the simulated

specimens, the Verigene did not detect 2/52 bla_{NDM} and 1/58 bla_{OXA} (again, a bla_{OXA-23}), whereas 68/68 bla_{VIM} and 47/47 bla_{IMP} were detected.[23] The second study that evaluated the Verigene® was performed across 3 hospitals in Japan, a country where IMP carbapenemases are prevalent. This study evaluated 141 positive blood cultures (1 that grew an IMP-producing isolate), and 205 blood cultures that were seeded with Gram-negative bacteria. 91 of these were CP-CRE, 84 of which were IMP variants. The Verigene® correctly detected all 92 CP-CRE, and no false-positive carbapenemase results were observed. However, 3 blood cultures yielded imipenem-resistant *Pseudomonas aeruginosa* isolates that were negative for carbapenemase genes, and 2 non-CP CRE (1 *Enterobacter* and 1 *K. pneumoniae*) in the seeded blood cultures were also predictably negative by the Verigene assay. As such, blood cultures with 'no carbapenem resistance gene detected' do not accurately predict efficacy of carbapenem therapy.[24]

Only one commercial, real-time PCR assay is in development for detection of CP-CRE from respiratory specimens. The Unyvero P55 is a multiplex PCR device manufactured by Curetis AG (Holzgerlingen, Germany) that detects 20 respiratory pathogens (19 bacteria, 1 fungus) and 17 drug resistance markers, directly from respiratory secretions in 4 hours.[25] Among the resistance markers are IMP, KPC, NDM, OXA and VIM genes. In a small study of 40 patients with pneumonia in a German hospital that evaluated an early version of the Unyvero test (P50 panel), 24 had a positive result by this assay. One of these patients had a bla_{KPC}-positive Unyvero result, but *Streptococcus pneumoniae* was the only bacterium detected by the Unyvero. In contrast, conventional culture of this patient's specimen revealed a *P. aeruginosa* isolate that was carbapenem-resistant and harbored bla_{KPC}. Further data are needed to assess the performance of this assay.[25]

Finally, several assays have been developed to detect carbapenemase genes directly from rectal swabs, to aid with rapid identification of colonized patients. The GeneXpert Carba-R assay is CE-IVD marked and recently achieved FDA clearance for use in the US[26] In addition, surveillance assays either in development or clinical trials include the Check-Direct CPE (Check-Points, Wageningen, Netherlands), RenDx Carbaplex assay (Renshaw, United Kingdom), and the Amplidiag CarbaR+VRE (Mobidiag, Espoo Finland). These assays detect a variety of carbapenemases, typically including the core KPC, NDM, VIM and OXA-48. While these methods allow rapid identification of patients colonized with CP-CRE, the major limitation identified to date for these PCR-based methods is low positive predictive values for some targets. Lowman and colleagues found a positive predictive value of only 46.6% for the detection of CP Gram negative bacteria in a study of 251 patients with rectal swabs performed using a laboratory-developed PCR.[27] Similarly, Lau and colleagues found a positive predictive value of only 21% in their study of the Check-Direct CPE for detection of CP-CRE among 258 patients evaluated at their institution.[28] In this latter study, 24/34 false positive results were due to detection of *blaVIM/NDM*, low-prevalence targets in most regions of the US, including Maryland, where the study was conducted. Indeed, the pre-test probability of a positive result for any carbapenemase is poor for most patient populations in the US, as the incidence of CP-CRE carriage remains low (<1 % in most cases). However, preselecting patients to be screened (e.g. those with history of treatment in long term care facilities or hospitalization overseas or those suspected to be part of an outbreak), may improve the performance of these tests.

Rapid non-nucleic acid based tests

To date, all non-nucleic acid based tests for the detection of CRE are performed on bacteria grown in pure culture (Table 3). The exception to this is the Accelerate Diagnostics method, which evaluates the response of bacteria in positive blood culture broth to carbapenem antibiotics, by automated microscopy. This method requires ~5 hours to perform, and in one study correctly classified 22/23 ertapenem-resistant *K. pneumoniae* and 24/24 ertapenem-susceptible *K. pneumoniae* isolates. Addition of 3-nitrophenylboronic acid, an inhibitor of the KPC enzyme, to the assay allowed correct classification of KPC among these isolates.[29] This test is not yet commercially available for patient testing, although clinical trials are under way.

The Carba NP is among the most widely used rapid, phenotypic carbapenemase detection tests performed by clinical and research laboratories. The method was developed by Patrice Nordman and Laurent Poirell in 2012,[30] to be performed from bacterial colonies. The method is based on the pH shift, detected by a phenol red indicator, that occurs concomitant with imipenem hydrolysis. The CarbaNP yields results within 2 hours, although some isolates yield a positive result much more quickly. The method performs very well with KPC and MBL producers.[30-33] However, the test has been demonstrated to have lower sensitivity for isolates that express β-lactamases with low imipenem hydrolysis activity, such as the OXA-48-like enzymes. One study that evaluated 13 class D oxacillinase producing Enterobacteriaceae found 4 false-negative results and 4 equivocal results, yielding an overall sensitivity of only 38.5% for this enzyme class.[33] Commercial versions of the Carba NP include the

RAPIDEC® CARBA NP (bioMerieux, Marcy L'Etoile) and the Rapid CARB Screen® (Rosco Diagnostica). Performance of these commercial systems appears to be on par with the manual method.[32,34,35]

Recently, Bogaerts and colleagues described the Bogaerts-Yunus-Glupcynski (BYG) Carba test, which is based on the same principles as the Carba NP, but uses an electrochemical method to detect imipenem hydrolysis. The BYG detects variations of conductivity in a polyaniline coated electrode that are imparted by the pH shift and redox activity that occurs during imipenem hydrolysis. The BYG method takes 5–15 minutes to perform. Preliminary data evaluating 324 isolates (178 CPs) suggest good sensitivity (97.2% compared to PCR) and specificity (100%). However, like the Carba NP, the BYG Carba test may yield false negative results for OXA-48-like carbapenemase producing isolates. 8/117 (6.8%) OXA-48-like carbapenemase producers were negative by this test, as was 1/13 VIM-2 isolates (7.7%) evaluated.[36]

Several groups have adapted matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI TOF MS), a tool used by many clinical laboratories for the identification of bacteria and fungi, to the detection of carbapenemase activity. These methods rely on incubation of the organism in question with a carbapenem, typically for 2–4 hours, and the mass-based detection of the carbapenem and its degradation productions. This is possible because hydrolysis of carbapenem β-lactam rings results in a measurable mass increase that can be differentiated from the native carbapenem mass on the MALDI TOF MS. Interestingly, longer incubation times are associated with false-negative results, possibly because the degradation products are further decomposed to a lighter mass that is not visible in the queried spectra of the assay.[37] Conversely, if the carbapenemase has only weak carbapenem hydrolytic activity, as is seen with the OXA-48-like enzymes, extensive incubation may be required (i.e. > 24 hours) before an appreciable change in the carbapenem spectrum is observed.[38,39] Additionally, some investigators have explored the use of quantitative MALDI-TOF to predict carbapenem susceptibility, by comparing peptide mass (a correlate of microbial growth), in the presence or absence of meropenem.[40] It is possible that further refinement of the method will yield improved results with universal testing conditions, but until that time, the MALDI-TOF MS is not a viable option for carbapenemase detection from isolates in the clinical laboratory.

While no phenotypic, direct-from-specimen methods are commercially available at present, several are under development, as described elsewhere.[41,42] Accelerate Diagnostics, Inc. is the only of these in clinical trials for direct, phenotypic susceptibility testing of bacteria in positive blood culture broths. The Accelerate method utilizes digital microscopy of immobilized live bacteria present in blood cultures to both identify (via fluorescent in-situ hybridization probes) and perform susceptibility (via observation of bacteria growth or inhibition in the presence of antimicrobials). Such technology would allow rapid detect of both CP and non-CP CRE within 6 hours of a blood culture flagging positive.

Treatment implications of rapid detection of CRE

Many observational studies report dismal outcomes among patients with CRE bloodstream infection, with mortality estimates ranging from 40% to nearly 70% among immunocompromised hosts.[43-45] These poor outcomes are due, in part, to patient comorbidities and severity of illness, as CRE disproportionally affect vulnerable patients (e.g.,, immunocompromised, critically ill and those with chronic medical issues) with substantial healthcare exposure.[46] Poor outcomes are also due to treatment with ineffective, empiric antibiotics before carbapenem resistance is identified by the laboratory.[47,48] In one study of neutropenic patients with CRE bloodstream infections, nearly 90% of patients were started on ineffective therapy at presentation and a median of 55 hours elapsed between time of culture collection and administration of effective antibiotic therapy.[45] Because mortality from sepsis increases 7.6% for every hour's delay in effective antibiotic administration,[49] diagnostics that speed up detection and appropriate treatment of CRE by even a few hours should improve outcomes of patients with invasive CRE infections. Earlier identification of patients with CRE infection should also lead to earlier consultation with Infectious Diseases (ID) specialists. ID consultation has been associated with favorable outcomes for patients with *S. aureus* bloodstream infections [50,51] and should similarly benefit patients with invasive CRE infections.

While the optimal therapy to treat invasive CRE infections has not been established, observational studies, mostly including patients with KPC-producing strains, suggest that mortality is lower when combination antibiotic therapy, rather than monotherapy, is used.[48,52-54] This remains controversial as not all studies found a survival advantage among patients with CRE infections who received combination therapy vs. monotherapy and results of ongoing randomized studies are not yet available.[55] There is some evidence that non-colistin based regimens had worse outcomes than colistin-based regimens.[56] Antibiotics that are active against CRE depend on carbapenemase type, but include polymyxins B and E

(colistin), tigecycline, fosfomycin, aminoglycosides, aztreonam, carbapenems (often administered at high doses using prolonged infusion), and the newly approved agent ceftazidime/avibactam.[57] Many of these drugs used in combination with each other or with adjunctive agents like rifampin or minocycline, demonstrate *in vitro* synergy against CRE.[58-62] Unfortunately, several of these CRE-active agents have narrow therapeutic windows and cause adverse effects, and their efficacy is reduced by emergence of resistance.[63,64] Outbreaks of colistin-resistant CRE have been reported in many regions,[65-68] likely due to increasing use of polymyxins for treatment of CRE. The prevalence of colistin resistance among CRE varies significantly across centers, but was as high as 22% in an Italian teaching hospital [65] and is approaching 50% in a large US tertiary care hospital (R. Humphries, unpublished). Alarmingly, polymixin resistance, which is usually due to mutations in several chromosomally-encoded genes, was recently found to occur through acquisition of a plasmid-mediated gene, *mcr-1*.[64] As such, molecular methods to detect carbapenemase genes cannot yet replace full susceptibility testing for the Enterobacteriaceae, as knowledge about susceptibility to a variety of non-carbapenem agents is needed to inform therapeutic decisions.

The type of carbapenemase present in CRE should influence choice of antibiotic therapy because carbapenemases vary in their hydrolyzing ability (Table 2). For example, metallo-β-lactamases like NDM do not hydrolyze the monobactam aztreonam, which may thus be effective against NDM-producing strains that do not also harbor aztreonam-hydrolyzing ESBLs. The combination of aztreonam-avibactam, currently in development, is anticipated to have good activity against strains that harbor both NDM and other β-lactamases, as avibactam inhibits the activity of ESBL and AmpC enzymes.[69] In contrast, OXA-48 carbapenemases are relatively inactive against cephalosporins and carbapenems; thus, carbapenems may be effective against OXA-48-producing strains that do not contain other β-lactamases. It has been suggested that carbapenems should be included in treatment regimens for OXA-48 containing strains that have low meropenem or imipenem MICs.[70,71] KPC and some OXA-48 carbapenemases are inhibited by ceftazidime-avibactam, which may be a therapeutic option for strains expressing these enzymes. However, this drug does not have activity against the class B metallo-β-lactamases (such as NDM),[72] and resistance in at least one KPC-producing *K. pneumoniae* has been described.[73] A few publications propose potential algorithms for treatment of CRE infection based on molecular detection of specific carbapenemases [74] and carbapenem MIC.[75,76]

Table 2. Carbapenemase types, hydrolyzing activity, and currently available active antimicrobial agents.

Carbapenemase	Hydrolyzing Activity	Active antibiotics[a]
KPC	Penicillins, narrow spectrum cephalosporins, extended spectrum cephalosporins, aztreonam, carbapenems	colistin/polymyxin B tigecycline[b] aminoglycosides[c] fosfomycin[c] rifampin[c] carbapenems[d] ceftazidime/avibactam
Metallo-β lactamases NDM, IMP, VIM	Penicillins, narrow spectrum cephalosporins, extended spectrum cephalosporins, aztreonam, carbapenems	colistin/polymyxin B tigecycline[b] aminoglycosides[c] fosfomycin[c] rifampin[c] carbapenems[d] aztreonam[e]
OXA-48, OXA-23	Penicillins, narrow spectrum cephalosporins, Weak activity against extended spectrum cephalosporins and carbapenems	colistin/polymyxin B tigecycline[b] aminoglycosides[c] fosfomycin[c] rifampin[c] carbapenems[d] aztreonam[e] 3rd generation cephalosporins[e] ceftazidime/avibactam

Adapted from.[74,75,76]
[a]Choice of therapy depends on *in vitro* susceptibility and site of infection. Combination therapy with multiple agents is recommended for critically ill patients.
[b]Consider high dose
[c]Adjunctive agents used in combination with other agents
[d]Generally given at high dose via prolonged or continuous infusion. Dual carbapenem-therapy can be considered for pan- resistant infections
[e]Isolates harboring other types of carbapenemases, ESBLs, or AmpC β-lactamases may not be susceptible

Infection control implications of rapid detection of CP-CRE

Timely identification of individuals who are infected or colonized with CP-CRE would enable prompt implementation of infection control interventions, including patient cohorting and isolation, precautions during invasive procedures and patient transport, and appropriate environmental disinfection. This, in turn, is likely to reduce transmission of CP-CRE within healthcare settings. Screening for CP-CRE colonization is a key component of CRE control as the number of individuals with intestinal CP-CRE colonization far outnumbers the few who develop CRE infection and are detected through clinical cultures. Knowledge of CRE colonization status could also lead to earlier effective empiric therapy for patients who are known to be CRE-colonized and may subsequently develop an infection.

The value of active surveillance for CRE colonization using point-of-care detection methods with rapid turnaround-time is illustrated by the Israeli response to a national CP-CRE outbreak due to dissemination of KPC-producing *K. pneumoniae* ST258.[77] In 2007, the

Table 3. Non-nucleic acid-based tests for detection of carbapenem resistance.

Test (Manufacturer)	Method	Specimen Type	Regulatory Status	References
Accelerate	Automated microscopy	Bacteria from positive blood culture broth	In development	29
Carba NP	Color indicator of imipenem hydrolysis	Bacteria from pure culture	Commercial versions have obtained CE-IVD	30-35
BYG Carba	Electrochemical indicator of imipenem hydrolysis	Bacteria from pure culture	Research use only	36
MALDI-TOF	Mass-based detection of carbapenem degradation products	Bacteria from pure culture	Research use only	37-40

Note. US. FDA, United States Food and Drug Administration; CE-IVD, Conformite Europeene *In Vitro* Diagnostic.

Israeli Ministry of Health instituted intensified infection control efforts in all acute care hospitals, including active surveillance for CP-CRE carrier status, and rapid cohorting of CP-CRE infected or colonized individuals in acute care facilities. Individuals at high risk for CP-CRE carriage (contacts of known CP-CRE carriers, patients on wards with high CP-CRE prevalence, transfers from other medical facilities) were screened for CP-CRE intestinal colonization using rectal swabs. CP-CRE colonization status was reported within 24 hours in order to enable prompt patient isolation and cohorting, if needed. Following implementation of these interventions, new CP-CRE carrier prevalence and the incidence of CP-CRE bacteremia decreased substantially.[77] Similarly, in the Chicago area, regional spread of a KPC-producing *K. pneumoniae* strain led to an outbreak that affected 40 patients and 26 healthcare facilities. This outbreak was ultimately controlled after implementation of a bundled intervention, including active surveillance of KPC, in several area hospitals and LTCFs.[78] Transmission of KPC-producing *K. pneumoniae* was also reduced in a New York City hospital through enhanced infection control including screening rectal surveillance cultures.[79] During a deadly outbreak of KPC *K. pneumoniae* at the National Institutes of Health Clinical Center in which 11 of 18 infected patients died, rectal surveillance cultures of all hospitalized patients and patient and staff cohorting were essential to halt the outbreak.[80,81] In this outbreak, the index patient had been discharged from the hospital 3 weeks prior to detection of the next CRE case, emphasizing the importance of rapid detection of asymptomatic CRE carriers who can propagate transmission.[81]

Additionally, it is important that surveillance methods be able to distinguish CP-CRE from strains with other mechanisms of carbapenem resistance because more aggressive infection control interventions are recommended for patients colonized with CP-CRE.[72] In control of the Israeli outbreak, those with CP-CRE were isolated and cohorted together while those with non-CP-producing CRE were placed in contact isolation, but were not cohorted. The US. CDC recognizes that facilities will vary in their CRE screening processes based on their regional CRE epidemiology, but has issued guidance that CRE screening be considered for patients who have had overnights stays in US facilities with high CRE prevalence, or in overseas healthcare facilities in the prior 6–12 months.[72] Despite this guidance, there remains great variability in CRE screening processes among US institutions. A survey of infectious disease providers and members of the Emerging Infections Network in 2014 found that only 18% of represented institutions performed active surveillance for CRE [82] Whether or not CRE screening is cost effective in settings with low CRE prevalence is unclear. In one US institution in a low CRE prevalence area, no CRE-colonized patients were identified after screening nearly 100 international, hospitalized patients using costly PCR- based methods.[83]

Implementation and reporting of rapid diagnostics for CRE

To optimize the clinical impact of rapid testing for CRE, there must be coordination between the clinical microbiology laboratory and antimicrobial stewardship and infection control programs for a number of reasons. First, selection of the appropriate platform for CRE detection should be based on local epidemiology and circulating carbapenemase types and should provide information that is likely to impact local clinical practice. Use of a platform that detects only bla_{KPC} in an institution where this carbapenemase is rarely found will not be useful. In a randomized single center clinical trial performed in a hospital with low CRE prevalence, use of the BioFire Blood Culture ID panel, which detects KPC as the sole Gram negative drug resistance marker, did not identify KPC in over 400 patients tested, and thus did not impact clinical management of Gram negative bacteremia.[84] Second, results of rapid testing must be communicated quickly to providers to enable timely clinical decision making. Third, the complexity of molecular testing for carbapenemases requires

interpretation by microbiologists, ID clinicians and/or antimicrobial stewardship teams because most clinicians will not understand the clinical significance of detecting specific resistance genes or discordance between phenotypic and genotypic resistance testing. Lastly, hospital epidemiologists and infection control staff must be promptly notified of patients infected or colonized with CRE so that appropriate infection control interventions can be implemented.

Rapid testing for CRE highlights the importance of clinical microbiology informatics, or the ability to integrate result reporting, communication to clinicians, and clinical decision support systems (CDSSs) in order to optimize patient outcomes.[85] CDSSs can take many forms, including comprehensive computerized disease management systems that can integrate clinical data, medication orders, and laboratory and microbiology results to suggest anti-infective therapy and alert clinicians about potential suboptimal management of infections.[86-89] Several studies of rapid blood culture diagnostics report favorable outcomes when rapid test results are reported together with CDSSs that provide actionable guidance for clinicians through antimicrobial stewardship team oversight [90-97] or even templated comments contained within the result report.[84] Increasingly, electronic health records platforms such as Epic and Cerner are incorporating CDSSs to enhance antimicrobial stewardship.[98] Implementation of rapid CRE diagnostics should be done together with CDSSs in order to improve outcomes for patients with invasive CRE infections, for whom there are often substantial delays in initiating effective antibiotics, with dire consequences.

Conclusions

Rapid diagnostics have the potential to improve surveillance, diagnosis, and treatment of CRE, an escalating public health threat, with limited treatment options and high mortality. A number of nucleic acid- and non-nucleic-acid-based methods for rapid detection of CRE are currently available or in development. While no molecular platform can contain all possible genes or resistance mechanisms conferring carbapenem resistance, one can imagine that future testing might incorporate rapid methods for both molecular detection of common carbapenemases and non-nucleic acid based determination of an isolate's overall antimicrobial susceptibility, enabling CRE to be identified promptly and effective antibiotic therapy and infection control precautions to be initiated within hours, rather than days, of a positive culture result. This, in turn would result in better outcomes, less transmission of CRE, less emergence of resistance, and lower healthcare costs. Molecular characterization of carbapenemase genes also offers the opportunity to target antibiotic therapy against specific resistance mechanisms, especially as new β-lactam/β-lactamase inhibitor combinations are developed, although further research is required to demonstrate this. To optimally impact patient outcomes, rapid testing for CRE should be implemented together with antimicrobial stewardship interventions or other forms of clinical decision support. The clinical and economic impact of rapid diagnostics for CRE identification will likely depend on local CRE prevalence and are areas for future research.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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