AMERICAN SOCIETY FOR MICROBIOLOGY | Clinical Microbiology Reviews

Intestinal Carriage of Carbapenemase-Producing Organisms: Current Status of Surveillance Methods

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SUMMARY

Carbapenemases have become a significant mechanism for broad-spectrum β-lactam resistance in *Enterobacteriaceae* and other Gram-negative bacteria such as *Pseudomonas* and *Acinetobacter* spp. Intestinal carriage of carbapenemase-producing organisms (CPOs) is an important source of transmission. Isolation of carriers is one strategy that can be used to limit the spread of these bacteria. In this review, we critically examine the clinical performance, advantages, and disadvantages of methods available for the detection of intestinal carriage of CPOs. Culture-based methods (Centers for Disease Control and Prevention [CDC] protocols, chromogenic media, specialized agars, and double-disk synergy tests) for detecting carriage of CPOs are convenient due to their ready availability and low cost, but their limited sensitivity and long turnaround time may not always be optimal for infection control practices. Contemporary nucleic acid amplification techniques (NAATs) such as real-time PCR, hybridization assays, loop-mediated isothermal amplification (LAMP), or a combined culture and NAAT approach may provide fast results and/or added sensitivity and specificity compared with culture-based methods. Infection control practitioners and clinical microbiologists should be aware of the strengths and limitations of available methods to determine the most suitable approach for their medical facility to fit their infection control needs.

INTRODUCTION

At some point, almost all *Enterobacteriaceae* were susceptible to broad-spectrum β-lactam antibiotics, including β-lactam–β-lactamase inhibitor combinations, oxyimino-cephalosporins (e.g., ceftriaxone, ceftazidime, and cefotaxime), aztreonam, and carbapenems. Regrettably, two seminal events occurred in the past 30 years, which have had a major impact on the therapy of infec-

Published 28 October 2015

Citation Viau R, Frank KM, Jacobs MR, Wilson B, Kaye K, Donskey CJ, Perez F, Endimiani A, Bonomo RA. 2016. Intestinal carriage of carbapenemase-producing organisms: current status of surveillance methods. Clin Microbiol Rev 29:1-27. doi:10.1128/CMR.00108-14.

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TABLE 1 Carbapenemases and selected characteristics[a]

Molecular class	Representative β-lactamase	Characteristic(s)	Inhibitor(s)	Enzyme currently found in areas of endemicity	Area(s) of endemicity
A	KPC, GES, SMC	Serine β-lactamase, plasmid encoded	Boronic acid derivatives	KPC	North America, Greece, Italy, Poland, Colombia, Argentina, Israel, China
				GES-5	Brazil
B	NDM, VIM, IMP, GIM-1, SPM	Metallo-β-lactamase, zinc requiring, plasmid encoded/chromosomal	EDTA, dipicolinic acid	NDM	Indian subcontinent, Kenya, China
				VIM	Indian subcontinent, Greece, Italy, southern France, Japan, Lebanon, Brazil, Portugal, Ireland, UK, Germany, Poland
				IMP	Indian subcontinent, Greece, Japan, China
C	CMY-10	Serine β-lactamase, cephalosporinases, mobile or chromosomal, uncommon	Cloxacillin, boronic acid derivatives	AmpC	Worldwide
D	OXA-48, OXA-181, OXA-204, OXA-162, OXA-23, OXA-24	Serine β-lactamases, weak activity of those that are carbapenemases, plasmid encoded	No specific inhibitors available	OXA-48	France, Belgium, Canada, South Africa, Middle East, Turkey, northern Africa, Switzerland, Germany, Lebanon, Israel, Morocco

[a] See references 16, 17, and 22.

tious diseases. In a manner analogous to the HIV epidemic and its human toll, the evolution of extended-spectrum β-lactamases (ESBLs) 3 decades ago significantly crippled the activity of oxyimino-cephalosporins and aztreonam, followed by the more recent appearance of carbapenemases in the clinic, which has limited the efficacy of all currently available β-lactams, causing a staggering economic and human burden (1). We have learned that increased colonization pressure from carbapenemase-producing organisms (CPOs) is linked to the development of infection (2), and gastrointestinal carriage of ESBL-producing *Enterobacteriaceae* leads to subsequent infection (3). Still today, after the initial report in 1983 of SHV-2 (the first ESBL reported), and despite significant advances in infection control and supportive care, infections caused by ESBL-producing *Enterobacteriaceae* exact an unacceptable mortality rate and add significantly to health care costs (4–6). The emergence of carbapenemases in the past 15 years has only added to the crisis caused by ESBL producers (7). The global impact of *Klebsiella pneumoniae* carbapenemase (KPC) and the New Delhi metallo-β-lactamase (NDM) created a worldwide fear that we are at the "end of the antibiotic era" (8, 9). The World Health Organization (WHO) has classified carbapenemase-producing *Enterobacteriaceae* (CPE) as one of the three greatest threats to human health (10). Surveys of the molecular epidemiology of carbapenemases, including KPC, OXA-48, VIM, IMP, and NDM producers, reveal that the dissemination of these carbapenemases is rapid and lasting. Authorities have advocated for local and regional screening programs, as available evidence shows that travelers are a major source of spread (11, 12). Furthermore, in settings where these organisms are endemic, transmission of ESBL-producing *Enterobacteriaceae* between health care facilities creates a significant challenge for controlling the spread of resistance (13). The number of CPE cases in community hospitals in the Southwestern United States has increased 5-fold in the last few years (14).

Current Status of Carbapenemases

Carbapenemases are present among all four classes of β-lactamases (Table 1) (15–17). A rare class C β-lactamase, CMY-10, also demonstrates weak "carbapenemase activity," but its clinical significance is unclear (17, 18). Additionally, CPOs are commonly resistant to multiple drug classes, such as aminoglycosides, quinolones, tetracyclines, and folate inhibitors, due to additional types of resistance genes carried by the organisms (19, 20). To provide the appropriate background for evaluating the detection methods discussed here, we review the major carbapenemases that are threatening our β-lactam arsenal.

Class A carbapenemases. One of the most common mechanisms of carbapenem resistance among class A enzymes is the production of KPC β-lactamases. KPCs were initially detected in a clinical isolate in 1996 in North Carolina; since then, 19 variants have been discovered (21–24). KPC has been found in a variety of *Enterobacteriaceae*, including *Klebsiella* spp., *Escherichia coli*, *Enterobacter* spp., *Citrobacter* spp., *Morganella* spp., *Serratia marcescens* (25–29), *Raoultella* spp. (30), *Kluyvera* (31), and *Salmonella* (32), and in non-*Enterobacteriaceae* such as *Aeromonas* (33), *Pseudomonas*, and *Acinetobacter baumannii* (34).

Attributable and crude mortality rates for infections caused by bacteria harboring KPCs are higher than those for patients with non-KPC-producing isolates (35); the reason for this increased mortality is still enigmatic. Epidemiological studies suggest that KPC-producing *K. pneumoniae* isolates belonging to sequence type 258 (ST258) are of two distinct clones and that the clinical behavior of isolates bearing bla_{KPC-2} is different from that of isolates carrying bla_{KPC-3}. Molecular differences between the two clones include aminoglycoside resistance and the ability to form biofilms (36, 37). The molecular reason for this difference in clinical behavior is not yet understood. The prevalences of KPC-producing bacteria vary widely. In one surveillance study, 37% of

patients in an intensive care unit (ICU) carried bla_{KPC} (38). Other studies place its prevalence at between 0 and 5%, depending on the population being surveyed (39, 40).

Some areas of Europe (Greece, Italy, and Poland), South America (Colombia and Argentina), the Middle East (Israel), and North America are areas where KPC is endemic. Recently, cases and localized outbreaks have been linked to importation from areas of endemicity (22, 41). In addition, long-term-care facilities (LTCFs) are rapidly becoming reservoirs for KPC producers (41). Other class A carbapenemases are important in some specific locales, such as GES-5 in Brazil, where it constitutes the main carbapenemase in *Enterobacteriaceae* (22). SME carbapenemases, also belonging to class A and associated with *Serratia marcescens*, are quite rare.

Class D carbapenemases. Another important carbapenemase in *Enterobacteriaceae* is a class D β-lactamase, OXA-48. This β-lactamase, sometimes referred to as the "phantom menace," was initially identified in a Turkish patient in 2001 (42–44). For the next 5 years, OXA-48 was not isolated from any other country. In 2008, OXA-48 spread outside Turkey and became prevalent in clinical isolates from Continental Europe, the Middle East, and northern Africa (45, 46). Since then, outbreaks throughout Europe have been reported (45, 47). Most recently, OXA-48 was detected in the United States, Canada, and South Africa (20, 48–50). Many of these reports involve patients previously treated in Middle Eastern and North African countries (51). Nonetheless, an early outbreak of OXA-48-producing *K. pneumoniae* in England was not linked to known regions of endemicity (52). More concerning, however, was a retrospective analysis that uncovered an outbreak of OXA-48-producing *Enterobacteriaceae* in a Dutch hospital that had been ongoing for 2 years (53). OXA-48 has disseminated to a wide variety of *Enterobacteriaceae* species, including *Klebsiella* spp., *E. coli*, *Citrobacter* spp., *Serratia marcescens* (54–56), *Enterobacter* spp., *Morganella morganii* (55), *Providencia stuartii* (57), *Raoultella planticola* (56), and *Salmonella enterica* (51).

OXA-48 is contained in a 61.8-kb self-conjugating IncL plasmid, which likely contributes to its ability to spread in *Enterobacteriaceae* (22, 58, 59). Other OXA-48-like enzymes with carbapenemase activity in *Enterobacteriaceae* that either have caused or have the potential to cause outbreaks include OXA-181, OXA-204, OXA-232, and OXA-162 (22, 43, 60–62). Other class D carbapenemases of clinical importance are OXA-23 and OXA-24/40; these carbapenemases are found mainly in *Acinetobacter baumannii* (63). Recently, some OXA-type carbapenemases have been reclassified based on their hydrolytic activity. To illustrate, once thought to be a carbapenemase, the kinetic profile of OXA-163 resembles more an ESBL than a carbapenemase (62).

Class B carbapenemases. The class B metallo-β-lactamases (MBLs) hydrolyze a broad range of β-lactams, including carbapenems (18). The most widespread MBLs include the NDM, VIM, and IMP family enzymes. Of the MBLs, NDM-1 has emerged as a major cause of concern due to its widespread dissemination (64). NDM-1 was initially detected in a patient of Indian origin in Sweden in 2007 (65). NDM-1 was subsequently found to be widespread in the Indian subcontinent, including in environmental samples (66), and has now been reported in more than 15 countries (67). In the United Kingdom, 52% of 101 patients with NDM-producing isolates collected from 2008 to 2013 reported health care exposure or travel to the Indian subcontinent (68). NDM has spread between different bacterial species, including *Enterobacter cloacae*, *K. pneumoniae*, and *Escherichia coli* (69).

Horizontal spread of NDM has also been described in the clinical setting; in a recent study, four neonates from India acquired an NDM-1-producing *E. coli* isolate from the environment and developed sepsis (70). Clonal spread of NDM-1-producing isolates has been documented in some regions of India, while spread elsewhere, including to the United Kingdom, likely happened due to a transfer of plasmids (71).

Equally important, IMP- and VIM-producing bacteria have also been found in the United States, Europe (mostly Greece, Italy, and southern France), the Middle East, the Indian subcontinent, Japan, and China (22, 72–74). Outbreaks have occurred throughout the world, as these MBLs spread as part of complicated integrons (42). To illustrate, a recent surveillance study performed in northeastern Ohio uncovered a clinical isolate of *Pseudomonas aeruginosa* with bla_{VIM-2} in a class I integron that was proximal to a *Salmonella* genomic island (SGI), suggesting recombination between these two bacteria. A detailed analysis of this genetic locus showed multiple resistance and transposing elements that likely resulted in the successful dissemination of this isolate (75).

MECHANISMS OF CARBAPENEM RESISTANCE

Resistance to carbapenems can be mediated by different mechanisms; these include porin mutations, upregulation of efflux pumps, changes in penicillin binding proteins (PBPs), and production of carbapenemases (76–78). A significant subset of carbapenemase genes are harbored in readily transmissible plasmids. These plasmids, in some circumstances, can be shared between *Enterobacteriaceae* and non-*Enterobacteriaceae*. While other mechanisms of resistance are also genetically encoded, their transmission is not as frequently observed as for carbapenemase genes and therefore are of lesser concern.

In this treatise, we generally refer to carbapenem-resistant organisms (CROs) as bacteria that are resistant to imipenem, meropenem, doripenem, or ertapenem. We particularly focus on Gram-negative CROs. These can be divided in *Enterobacteriaceae* and non-*Enterobacteriaceae*. Carbapenem-resistant *Enterobacteriaceae* are frequently referred to as CRE. Organisms that are carbapenem resistant due to the production of a carbapenemase are referred as carbapenemase-producing organisms (CPOs), and when the bacteria are *Enterobacteriaceae*, we refer to them as carbapenemase-producing *Enterobacteriaceae* (CPE). In addition, there are some bacteria that produce carbapenemases even though their MICs for carbapenems do not reach the resistance breakpoint. Given that carbapenemase genes are usually transmissible via plasmids, we argue that they should be targeted for screening, and we include them as CPOs or CPE. It must be noted that some non-*Enterobacteriaceae* CPOs such as *Burkholderia* spp. and *Stenotrophomonas maltophilia* carry chromosomally encoded carbapenemases. As such, the chromosomally encoded carbapenemases are unlikely to be transmitted to other bacteria. When we refer to CPOs in this review, we focus on all *Enterobacteriaceae* and the non-*Enterobacteriaceae* that are known to carry carbapenemase-encoding plasmids, even when their MIC increase does not reach the resistance breakpoint.

INTESTINAL CARRIAGE OF CPOs

As noted above, CPOs have emerged as significant health care-associated pathogens worldwide (38, 79). While most studies refer

TABLE 2 Clinical breakpoints for carbapenems according to CLSI and EUCAST guidelines[a]

Carbapenem	Susceptibility breakpoint (μg/ml) according to:			
	CLSI document M100-S20, 2010 (196)	CLSI document M100-S20U, 2010 (197)	CLSI document M100-S22, 2012 (198)	EUCAST, 2009–2014
Doripenem	Not defined	≤1	≤1	≤1
Ertapenem	≤2	≤0.25	≤0.5	≤0.5
Meropenem	≤4	≤1	≤1	≤2
Imipenem	≤4	≤1	≤1	≤2

[a] An intermediate result is interpreted as an MIC that is 1 dilution higher, and a resistant result is interpreted as an MIC ≥2 dilutions higher, except for the EUCAST interpretation of ertapenem results, where an organism with an MIC of >1 μg/ml is considered resistant. CLSI documents M100-S23 (199) and M100-S24 (200) (2013 and 2014) do not change the interpretative criteria for carbapenems. CLSI document M100-S22 (2012) (198) changed interpretative criteria only for ertapenem. The criteria for other carbapenems were not changed. Doripenem was not included in the 2009 and 2010 editions of CLSI document M100.

to CPE, we contend that similar conclusions can be applied to CPOs, encompassing both *Enterobacteriaceae* and nonfermenting Gram-negative bacteria. Furthermore, since carbapenemases are transferred via plasmids, both *Enterobacteriaceae* and non-*Enterobacteriaceae* are capable of serving as reservoirs and vectors. Intestinal carriage serves as a reservoir of CPE and can promote cross-transmission in health care settings (80). Thus, infection control programs directed at detecting intestinal carriage are essential tools to limit the spread of these pathogens.

Several examples highlight the importance of detection of intestinal carriage for the effective control of infections due to CPOs. A study in New York documented a significant decrease in the carriage rate 1 year after an infection control program in an ICU was implemented (81). The program involved screening for intestinal carriage of carbapenem-resistant *K. pneumoniae* and *Acinetobacter baumannii* with culture of rectal swabs (BBL CultureSwab Plus; Becton-Dickinson) and isolating patients while results were pending or if they were positive. Isolation was carried out in the rooms at the far end of an ICU, where rooms were divided only by curtains. The program also involved extensive cleaning with isopropanol and a quaternary ammonium compound, which included closing the unit for 2 days. The lack of a quick screening test was a limiting factor for the success of this program. Nonetheless, those investigators were able to reduce the mean number of new KPC-producing *K. pneumoniae* cases from 9.7 to 3.7 per 1,000 patient-days. In another study, Enfield et al. were successful in decreasing the CPE incidence in a surgical ICU from 7.77 cases per 1,000 patient-days to 1.22 cases per 1,000 patient-days by using enhanced infection control measures and increased surveillance by implementing a PCR-based assay (82). Two outbreaks of KPC-producing organisms were successfully controlled by using a "bundle approach," of which screening for CPE carriage is an integral part (83, 84). Schwaber et al. reported on a country-wide mandatory program involving physical isolation and dedicated nursing staff in Israel that was able to significantly decrease the incidence of KPC-producing isolates (85). Although screening for asymptomatic carriage was not part of the program, this effort involved a very broad isolation policy that relied on careful tracking of known cases throughout the health care system. In addition to these real-life examples, a mathematical model also validates the usefulness of screening followed by patient isolation to control CPOs (86).

Failure of Carbapenem Breakpoints To Detect All CPOs

Detection of CPOs in the clinical microbiology laboratory is challenging because interpretation of routine susceptibility test results may fail to flag an isolate as a potential CPO (14, 87–89). Although the presence of a carbapenemase confers some resistance, the increase in the MICs due to the β-lactamase may not be enough to consider the isolate resistant to a carbapenem given the defined cutoff values for interpretation of resistance (90). Despite the changes made by the Clinical and Laboratory Standards Institute (CLSI) to the carbapenem interpretative criteria for *Enterobacteriaceae* in June 2010, which lowered the MIC values for isolates that were considered "nonsusceptible" (from ≤4 μg/ml to ≤1 μg/ml for meropenem) to detect more CPE than under previous guidelines, there are some isolates that still escape detection (90). Recently, both the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the CLSI have proposed not testing for resistance mechanisms in clinical isolates, arguing that the lower breakpoints should suffice for treatment purposes (Table 2) (91, 92).

We must emphasize that clinical breakpoints are meant for implementation in the care of patients and are not designed for epidemiological surveillance. In any case, uniform consensus on this issue does not exist; we agree with Livermore et al. and advocate for testing for carbapenemase resistance genes for infection control monitoring as well as for routine microbiological diagnosis (93). The rationale for this assertion is that although increased MICs against carbapenems may suggest the presence of a CPO, clinical experience demonstrates that MICs will not always reveal the presence of carbapenemases. The EUCAST proposed the use of epidemiological breakpoints followed by phenotypic confirmation by inhibition disks or the Carba NP test for this purpose (94). All of these changes reflect the notion that to prevent the spread of resistance, it is necessary to prevent the transmission of not only isolates that are phenotypically resistant but also those that carry transmissible elements that may spread to susceptible bacteria to confer resistance under the right conditions.

Carbapenemases, when not accompanied by other β-lactamases, may confer a low level of resistance to carbapenems (even MICs of ≤0.5 μg/ml) that does not become evident until they are combined with another resistance mechanism, such as the production of an ESBL or acquired AmpC (95, 96), porin mutations (97, 98), or changes in porin expression (99). Conversely, these changes may increase carbapenem MICs in the absence of carbapenemases. OXA-48 is particularly known for consistently failing to be detected if not accompanied by another broad-spectrum β-lactamase (100). This was demonstrated in the unrecognized Dutch outbreak described above (53).

Reports show that automated susceptibility systems are also not

reliable for the detection of carbapenemase-producing *K. pneumoniae* isolates (101). According the interpretative criteria for meropenem from the 2005 CLSI document M100-S15 (which corresponds to document M100-S22, 2012, in Table 2), rates of nonsusceptible isolates in a panel of confirmed KPC producers ranged from 93% with the Microscan system (Beckman Coulter, Brea, CA, USA) to 20% with Sensititre AutoReader (Thermo Scientific, Waltham, MA, USA), compared with 100% for broth microdilution and disk diffusion (102). For KPC-producing non-*Klebsiella* isolates, the rate of false-negative results may be higher, although there has been some improvement using the revised CLSI breakpoints (88). The use of stricter criteria and expert rules for automated systems has increased the sensitivity of CPE detection but with a significant decline in specificity (103). A study comparing disk diffusion, Etest, and Vitek2 (bioMérieux, Marcy l'Etoile, France) methods using previous CLSI and EUCAST breakpoints for meropenem, imipenem, or ertapenem found multiple discrepancies with KPC, ESBL, and MBL producers. However, the Vitek2 system, when using meropenem as a reporter substrate, successfully detected all CPE producers (87).

On an operational basis, ertapenem and meropenem are proposed to be the most suitable antibiotics for screening of carbapenemase producers (89). Anderson et al. found that, depending on the method used, 0 to 6% of KPC-producing isolates were susceptible to ertapenem according to the former CLSI ertapenem resistance breakpoint of ≥8 μg/ml. After the breakpoint was decreased to ≤2 μg/ml (which is higher than the current breakpoint of 0.5 μg/ml), almost all tested methods were able to detect 100% of the KPC producers. Interestingly, the Vitek2 platform, when using ertapenem as an indicator, still failed to detect 6% of these isolates (89). The EUCAST guidelines reflect these issues, suggesting that meropenem (with a cutoff of 0.125 μg/ml) is the antibiotic with the best balance between sensitivity and specificity while noting that ertapenem is the most sensitive but lacks specificity (94). Faropenem, a penem antibiotic, has also been proposed as an alternative to carbapenems for the detection of carbapenemases (104, 105). Faropenem showed 99% sensitivity and 94% specificity when tested against a known panel of 166 PCR-confirmed isolates of carbapenemase-producing *Enterobacteriaceae* (although OXA-48-producing isolates were underrepresented) and 82 negative controls. Another study compared a panel of 62 PCR-confirmed KPC-producing *Enterobacteriaceae* and 73 producers of other β-lactamases, showing a nonoverlapping inhibitory zone around a 5-μg faropenem disk between KPC producers and nonproducers (104).

Tests for Carbapenemase Activity in Isolated Cultures

Tests that detect carbapenemase activity in isolated cultures within a short time period can be used to rapidly determine if a clinical isolate is a CPO. These tests are generally not suited for direct testing of nonsterile specimens without prior isolation or enrichment steps, so they would not be used to screen fecal specimens or perirectal swabs directly. However, they can be employed as confirmatory assays when using culture-based screening.

The modified Hodge test (mHT) was the initial screening test recommended for carbapenemase production (89). However, mHT lacks specificity and may produce false-positive results for bacteria with complex ESBL or AmpC (both plasmid and overexpressed chromosomal enzymes) backgrounds combined with porin mutations/loss (106, 107). For the mHT, it must be noted that this test should be performed with either meropenem or ertapenem, as it is known to perform poorly when imipenem is used as a substrate (92). When focusing on KPC enzymes, the specificity of this test can be increased by using an EDTA disk, as described by Yan et al. (108); however, the most common reason for false-positive results (i.e., AmpC hyperproduction) is not addressed with this modification. The mHT is also unable to distinguish between carbapenemases, and it lacks sensitivity for some carbapenemases such as NDM (particularly low-level producers), some members of the OXA family, and SME (107, 109).

Synergy testing with inhibitors can be used to differentiate MBLs from other enzymes by inhibiting class A, C, and D enzymes. The phenylboronic acid double-disk synergy test (PBA-DDST) with either meropenem or ertapenem was able to successfully screen for KPC β-lactamase in a collection of clinical specimens (110). Pournaras et al. evaluated PBA and EDTA with meropenem in disks in a sample of bacterial colonies isolated from 189 rectal swabs where 97 were positive for a carbapenemase (KPC and VIM) and showed excellent sensitivity and specificity (111). Doi et al. also showed that inhibition by PBA could be used to differentiate KPC from other β-lactamases (112).

Another proposed improvement to disk-based testing that allows differentiation between the different β-lactamase classes is the use of avibactam (formerly NXL104) disks (113, 114). Class C enzymes can also be identified with similar inhibition tests (115, 116). Further improvement of these tests allows the identification of concurrent mechanisms, such as KPC with a MBL, such as the one suggested by Miriagou et al. (117). In that study, PBA improved MBL inhibition, allowing fewer misclassifications of VIM and KPC than with the use of PBA. These disk tests, however, require pure cultures, making them inappropriate for screening of the lower gastrointestinal tract of patients. Nevertheless, these assays remain a low-cost, low-technology option.

The Carba NP test, the Blue-Carba test, and the Rapid Carb test are based on the detection of carbapenemase activity. The Carba NP test (bioMérieux, France) detects a change in pH that is coupled to the hydrolysis of imipenem. The testing procedure consists of cell lysis followed by incubation of the enzymatic lysate with imipenem and phenol red for up to 2 h (118–120). Testing of *Enterobacteriaceae* showed that Carba NP was able to detect all tested isolates from a worldwide collection of bacteria that produced class A (KPC, NMC, SME, and GES), class B (IMI, NDM, VIM, and IMP), and class D (OXA-48 or OXA-181) enzymes (119). In this report, false-positive results were not detected. The Carba NP test has also been used directly on blood culture bottles spiked with an array of class A, B, and D carbapenemases, where this assay demonstrated a sensitivity and a specificity of 97.9% and 100%, respectively (121). However, in another study, the Carba NP test produced noninterpretable results for testing of isolates grown on MacConkey or Drigalski agar (120), suggesting that these media may affect the performance of the test. Also, the use of the Carba NP test on *A. baumannii* may require some modifications (122). In any case, the Carba NP test is one of the recommended tests for confirmation of carbapenemase production in pure isolates by the CLSI and EUCAST (92, 94).

The Carba NP test can be performed in most microbiology laboratories, with no additional equipment. It can be used on any isolate with suspected carbapenemase activity. The main advantage over agar screening and molecular methods is the broad target range, as the test result will be positive as long as there is

enough carbapenemase, regardless of its class. However, there is some concern regarding the lack of sensitivity for certain class D carbapenemases (e.g., OXA-48, OXA-58, and OXA-181) (123, 124). A possible drawback compared to molecular methods (and disk inhibition testing) is its inability to differentiate between enzymes, although one report suggests that this can be achieved with some modifications to the Carba NP test (125). The Carba NP test has also been compared to an alternative colorimetric test for carbapenemase activity, the Rapid Carb Screen (Rosco Diagnostics, Denmark), showing a similar sensitivity (97 and 98%) but superior specificity (100 and 83%) when tested against a panel of 66 *Enterobacteriaceae* isolates carrying class A, B, or D enzymes and 69 non-carbapenemase producers (126). Another test that is similar to the Carba NP test, the Blue-Carba test (Rapid Carba Screen; Rosco Diagnostics), uses a different indicator and a simplified protocol. The Blue-Carba test has a higher reported sensitivity, including for OXA-type carbapenemases (127). An added advantage of the Blue-Carba test is a faster turnaround time than that of the Carba NP test, as there is no need to extract the β-lactamase.

Spectrometry has also been used to detect CPOs. These tests include UV spectrophotometry and mass spectrometry using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS). UV spectrophotometry involves the detection of hydrolyzed imipenem by a cell lysate (128). This method was found to be 100% sensitive and specific for detecting a wide array of class A, B, and D enzymes in *Enterobacteriaceae* (129). The clinical application of this method is still challenging due to the technical expertise and equipment required to perform it.

MALDI-TOF MS can detect carbapenemases by comparing the proportions of hydrolyzed and intact imipenem in a centrifuged cell sample (130). This approach was able to detect 72% of carbapenemase-producing isolates directly from positive blood culture vials (131). Although still a research application, this method may become attractive as MALDI-TOF MS becomes more common in the microbiology laboratory, but this method is suboptimal for the detection of small molecules, such as carbapenems and their degradation products. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is more suitable for this task and has also been used successfully to detect carbapenemase activity from cultures (132). These methods have the potential to differentiate between classes of β-lactamases by using inhibitors such as EDTA (133). It must be noted that imipenem undergoes spontaneous hydrolysis in basic buffers (134) and that a negative control should always be included when using any of these methods. At this time, a mass spectrometry method that is as sensitive and easily implemented as agar-based or PCR-based screens is not yet present.

Screening Methods To Detect Fecal Carriage of CPOs

Screening tests to detect CPOs in stool present three major challenges: rapid detection, detection of isolates with low-level carbapenem resistance, and detection of proportionally low numbers of CPOs. Infection control programs rely on contact isolation for patients who test positive, which also must be in place while waiting for the result. A "good screening test" must minimize turnaround time, maximize sensitivity, preserve reasonable specificity, detect multiple types of carbapenemases, and be cost-effective. Detection of low-level resistance is important because it may already signify the presence of a genetic trait (such as bla_{KPC}) (90) that may spread to other bacteria through horizontal transfer, where it could result in carbapenem resistance in new bacterial strains (135). Finally, since the main reservoir is the intestinal tract, the bacteria of concern may represent just a small proportion of the overall bacterial load. Therefore, the inoculum of CPOs on a surveillance swab may be below the limit of detection (LOD). It is also worth mentioning that non-*Enterobacteriaceae* Gram-negative bacteria may also harbor carbapenemases, although screening protocols based on culture methods try to exclude them. For instance, U.S. Centers for Disease Control and Prevention (CDC) protocols and the suggested interpretation for the Supercarba agar test suggest that only lactose-fermenting colonies should be reported (see Fig. 1). Manufacturers of chromogenic media also endorse the reporting of colonies with certain appearances that correspond to lactose fermenters. Bacteria other than lactose-fermenting *Enterobacteriaceae* can be detected with appropriate culture methods, and molecular screening tests will yield a positive result if a carbapenemase is present, regardless of the host organism. Inclusion of bacteria other than lactose fermenters in a screening program is important, as they can also transmit resistance elements to or within the *Enterobacteriaceae*, as has been previously suggested (75).

A summary of tests is provided in Table 3. As shown, the cost, labor intensity, and turnaround time vary by assay. Mathers et al. reported that the annual costs of a surveillance program for a hospital containing 708 acute-care beds and 40 long-term beds with weekly screening and a CPE prevalence of 2.7% were about $225,000 for a qPCR (quantitative real-time PCR) assay and $23,000 for the CDC screening culture method (136). Although Mathers et al. accounted for the cost of decreased specificity, the cost of decreased sensitivity is much more difficult to calculate. For instance, a false-positive result (product of low specificity) would result in further follow-up testing; however, a false-negative result (product of low sensitivity) may result in the spread of the CPO, potentially adding very significant costs for the hospital to care for infected patients, while instead, it would appear to decrease the cost of the screening program. The apparent difference between costs of methods can translate into many thousands of dollars per year for a hospital performing routine screening in large volumes. Added to the cost of screening is the cost of isolation. A 2014 Canadian study estimated a cost of Can$925 (approximately US$740) per non-ICU patient when a patient is isolated for 3 days while awaiting results (137).

Culture-based methods. Culture-based testing is easier to implement, as the necessary equipment and knowledge are already present in the routine microbiology laboratory. These tests also have the potential to detect reduced susceptibility to carbapenems caused by newly emerging mechanisms as long as the mechanism is able to achieve at least a moderate level of resistance.

The CDC screening method addresses, with significant limitations, the need for the detection of low-level resistance (MIC < 2 mg/liter) and the ability to detect low loads of resistant bacteria. This method consists of an enrichment phase where a rectal swab is inoculated into 5 ml of Trypticase soy broth (TSB) in which a disk impregnated with 10 μg of ertapenem or meropenem has been immersed and incubated for 24 h. This broth is then subcultured onto MacConkey agar, where only lactose fermenters are selected. The CDC notes that many laboratories add a meropenem or ertapenem disk to this agar. A limitation of this test is that further testing is needed to determine the species and antimicro-

TABLE 3 Characteristics and approximate costs of screening methods to identify fecal carriage of CPE

Method (reference)	Description	Turnaround time for positive or preliminary positive result (h)	Price (US$)[a]
CDC protocol (138)	Broth enrichment of rectal swab in ertapenem medium followed by subculture on MacConkey agar with carbapenem disk, followed by identification of suspect isolates	48–72[e]	Negative test result, 1–2[b]; positive test result, 2–6[b]
Supercarba (149)	Direct plating of rectal swab in selective medium	24–48[e]	1[c]
Chromogenic medium	Direct plating of rectal swab in selective medium with chromogenic molecule	24–48[e]	4–7
Real-time PCR[d] (in-house methods)	DNA extraction followed by PCR and probe-based detection	2–5	10–30[b]
Commercial PCR assay[d]	DNA extraction followed by PCR and probe-based detection.	2–3 possible	30–60[b]

[a] U.S. dollars as of 2015.
[b] Based on cost data by Mathers et al. (136).
[c] Supercarba medium has been patented. The cost is that of raw materials; this medium is not available to many laboratories.
[d] The cost of the PCR assay may increase with increased numbers of targets.
[e] Confirmation testing might include singleplex PCR, multiplex PCR, Carba NP, Blue-Carba, or identification and susceptibility testing. The cost may range from an additional $2 to $50, and the turnaround time may range from an additional 2 to 24 h for confirmatory testing, depending on the methods chosen by the laboratory. Hospital epidemiology can act on negative results and preliminary positive results, pending confirmation. Negative NAAT results likely do not require confirmatory testing, but positive results may require confirmation, depending on the false-positive rate of the assay.

bial susceptibility of isolates growing on the agar (138). Furthermore, bacteria other than lactose fermenters that can harbor carbapenemases are routinely missed. Given the increased length of time needed for detection when using methods such as the CDC method, selective agars (see below) have been developed to optimize detection while obtaining results in a shorter time span. More important is that the CDC method will fail to detect the presence of bacteria with low-level resistance unless these bacteria are present in a large inoculum and without competition with other CROs, conditions that are unlikely to happen. Furthermore, small inocula of fully resistant CPOs can be missed if there is a large inoculum of bacteria that have low-level carbapenem resistance through mechanisms other than carbapenemases. Although the CDC broth enrichment method was meant to increase sensitivity, recent reports demonstrate that some of the selective agar methods have a performance that is superior or at least comparable to that of the CDC method, so the delay of an overnight enrichment is not necessary (139–141).

Specialized solid media aim to simplify the detection of CPE. Chromogenic media incorporate chromogenic enzyme substrates (mainly glycosides) that release a pigment when hydrolyzed by bacterial enzymes (142). Antibiotics added to the media make them selective for a particular resistance trait. Chromogenic media have been compared regarding their limit of detection of CPE with different inocula when used for stool screening (80, 139, 143–146). At this time, the currently available media have not been cleared by the U.S. Food and Drug Administration (FDA).

Available chromogenic media that may be used for the detection of carbapenemases include CHROMagar KPC (CHROMagar, France), HardyChrom (Hardy Diagnostics, CA, USA), chromID Carba (bioMérieux, France) (Fig. 1), chromID ESBL (bioMérieux, France), chromID OXA-48 (bioMérieux, France), Colorex KPC (Biomed Diagnostics, OR, USA), RambaChrom KPC (Gibson Bioscience, USA), SpectraCRE (Thermo Diagnostics, USA), and Brilliance CRE (Thermo Diagnostics, USA). Colorex KPC medium consists of medium commercially prepared from dry CHROMagar reagents.

Some of these media are designed to target KPC producers and

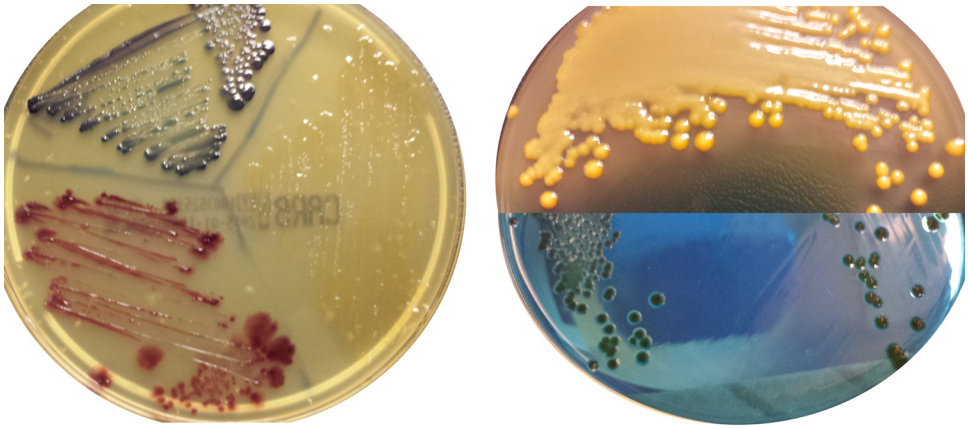

FIG 1 Appearances of different *Enterobacteriaceae* on chromID Carba and Supercarba media. (Left) chromID Carba plate. Red colonies represent *K. pneumoniae*, blue colonies represent *E. coli*, and yellow colonies represent *Pseudomonas aeruginosa*. (Right) Supercarba medium composite picture. The top half shows *K. pneumoniae* (yellow colonies due to lactose fermentation). The bottom half shows *Pseudomonas aeruginosa* (black/dark green colonies with no lactose fermentation).

TABLE 4 Use of known resistant isolates to test performance of CPE screening methods[a]

Method	Overall sensitivity (%)	Sensitivity (%) by β-lactamase class (no. of isolates tested)			Specificity (%) (no. of negative isolates tested	Type(s) of isolates	Reference
		Class A	Class B	Class D			
Supercarba	95.6	100 (18)	90 (52)	100 (44)	82.2 (62)	CLI	149
chromID ESBL	87.7	100 (18)	98 (52)	70 (44)	24.2 (62)		
CHROMagar KPC	40.3	66.7 (18)	55.8 (52)	13.6 (44)	85.5 (62)		
Supercarba	96.5	100 (20)	92 (51)	100 (43)	60.7 (28)	CLI	143
CHROMagar KPC	43	70 (20)	58.8 (51)	11.6 (43)	67.8 (28)		
Brilliance CRE	76.3	85 (20)	78.4 (51)	69.8 (43)	57.1 (28)		
Brilliance CRE	86	100 (17)	72 (25)	88 (58)	40 (77)	CLI	147
Colorex KPC	48	100 (17)	52 (25)	31 (58)	39 (77)		
Supercarba	97	100 (17)	88 (25)	100 (58)	35 (77)		
Brilliance CRE	78	83 (12)	79 (103)	67 (15)	66 (70)	CLI	144
chromID Carba	91	100 (12)	93 (103)	67 (15)	89 (70)		
chromID ESBL	96	100 (12)	98 (103)	80 (15)	19 (70)		
Colorex KPC	56	83 (12)	52 (103)	60 (15)	77 (70)		
CDC protocol for ertapenem	78	83 (12)	80 (103)	73 (15)	69 (70)		
CDC protocol for meropenem	47	67 (12)	46 (103)	40 (15)	79 (70)		
Brilliance CRE	94	100 (36)	94 (34)	84 (25)	71 (160)	CLI, CCI	146
mHT	100	100 (18)	ND (0)	ND (0)	96.7 (32)	CCI	110
RambaChrom KPC	95	95 (18)	ND (0)	ND (0)	77.1 (32)		
Mero-PBA-DDST	100	100 (18)	ND (0)	ND (0)	100 (32)		
Erta-PBA-DDST	100	100 (18)	ND (0)	ND (0)	91.4 (32)		

[a] CCI (characterized clinical isolate) is an isolate originating from a clinical specimen and later characterized in the laboratory. CLI (characterized laboratory isolate) is an isolate retrieved from a laboratory source. It may have originated from a clinical specimen but may have been modified to express certain characteristics in the laboratory. ND, not determined; mHT, modified Hodge test; PBA-DDST, phenylboronic acid double-disk synergy test; Mero, meropenem; Erta, ertapenem.

have markedly decreased sensitivity for mechanisms based on other enzymes, particularly OXA-48 (143, 147). This is specifically addressed with a medium designed for the detection of OXA-48 producers, chromID OXA-48.

Table 4 shows the performance characteristics of different chromogenic media when tested with pure cultures. The specificity varies depending on the type of negative controls used (clinical specimens or known non-carbapenemase-producing but carbapenem-resistant isolates). In addition, although not shown in Table 4, all testing methods had slightly but consistently lower sensitivities for VIM β-lactamases than for other class B β-lactamases (80, 139, 143–146). This may be due to the inclusion of isolates that contained plasmids carrying at most another β-lactamase, rather than isolates with more complex backgrounds that have now become prevalent (148). However, this likely does not hold true for bacteria harboring VIM-containing plasmids that also carry an ESBL or another carbapenemase.

Supercarba agar (Fig. 1) is another specialized medium that incorporates the use of ertapenem (0.5 mg/liter) in addition to cloxacillin in a zinc-supplemented Drigalski lactose agar (149). Ertapenem will select for carbapenem resistance, and cloxacillin is added to inhibit the growth of AmpC producers such as *Serratia* and *Enterobacter* species, while zinc enhances the activity of MBLs (149). Different studies have shown a sensitivity of ~96% and a specificity of 60%. These numbers are similar to those obtained with chromogenic media (143, 149). Those authors recommend the selection of only lactose-fermenting bacteria, limiting the ability of this method to detect carbapenemases in bacteria other than lactose fermenters. Another disadvantage is that the shelf life of the medium is limited to 7 days, a significant obstacle in any routine clinical laboratory (143).

Relative performance of culture methods. Studies describing different methods for screening of CPE are difficult to compare, and each study has its own limitations and particular variations. Some studies have addressed the detection limit of different commercial assays by using previously characterized CPE isolates. These isolates, however, may not be representative of the population at a specific hospital, and clinical performance in actual practice may vary due to the prevalence of different β-lactamases in different institutions. Meanwhile, other studies have compared the performances of tests in a particular setting, such as hospitals, where there is a particular distribution of resistance mechanisms within the bacterial population. It is difficult to extrapolate the performance of these tests to other clinical settings. In addition, some studies use comparators that are known to perform poorly, which may exaggerate the performance of certain media.

Performance characteristics of the different media used for screening of rectal or perirectal swabs are shown in Table 6. Of the 13 studies mentioned, 9 showed an almost exclusive presence of KPC producers (80, 139, 141, 150–156), while 2 revealed the exclusive presence of NDM producers (157, 158). Only two studies were done at institutions where KPC and VIM producers were reported to coexist (140, 159), and only one study was performed with OXA-48-producing isolates (160). Furthermore, the prevalence of ESBL producers at these locations is not taken into account and could impact the specificity of these screening methods.

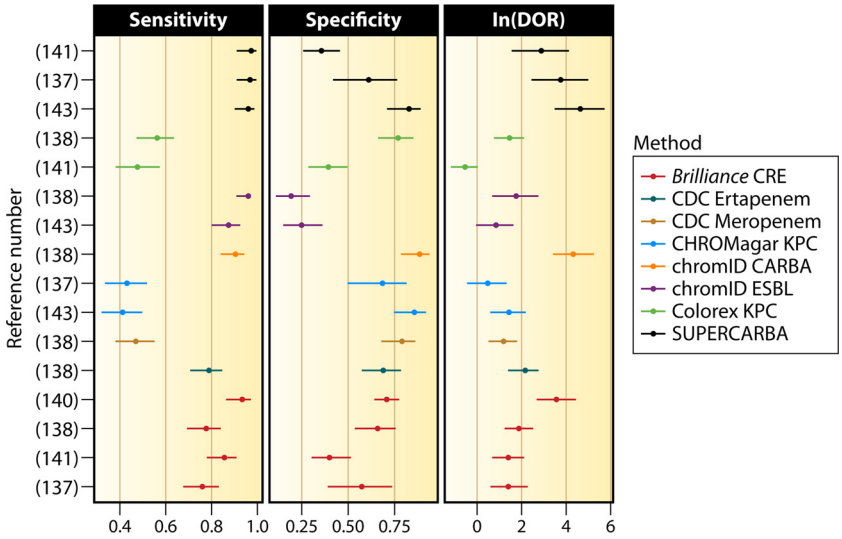

FIG 2 Per-observation estimates of sensitivity, specificity, and DOR for screening methods used on pure cultures included in statistical analyses (137, 138, 140, 141, 143).

The different screening systems performed variably on stool specimens compared with pure cultures (Table 4), showing mostly declines of both sensitivity and specificity with stool specimens.

We assert here that the sensitivity of a screening medium corresponds to the sum of sensitivities for each particular mechanism (e.g., OXA-48, KPN, and NDM). If a particular medium is tested in cases where one mechanism is overrepresented, it will have a greater contribution to the calculated sensitivity for the detection of CPE. For instance, consider that medium A has sensitivities of 90% for KPC and 70% for OXA-48. If this medium is tested where 95% of CPE are KPC producers while 5% are OXA-48 producers, the study will show an overall sensitivity for CPE detection of 89%. However, if 70% of CPE are OXA-48 producers and 30% are KPC producers, it will show an overall sensitivity of 76%.

To place our analysis in a clinical perspective, we performed a statistical analysis comparing the sensitivities, specificities, and diagnostic odds ratios (DORs) of the different methods used for screening of pure cultures by employing a bivariate random-effects model (161) using the mada package of the R programming language (162, 163). The DOR is the ratio of the odds of the test producing a true-positive result to the odds of it producing a false-positive result. The bivariate random-effects model is a meta-analysis technique for pooling diagnostic performance measures across studies and estimating covariate effects. The corresponding forest plots were generated with ggplot2 (164). Data from methods that did not detect all three carbapenemase classes were excluded. Figures 2 and 3 illustrate the sensitivities, specificities, and DORs for the different media in each study and in aggregate, respectively. Table 5 shows the model-estimated 95% confidence intervals for these parameters. Given the proportion of class B carbapenemase-producing isolates included in these studies, their effect on the estimated pooled performance characteristics is likely disproportionate. The same approach was used to analyze performance on rectal/perirectal swabs (Table 6). Given the low number of specimens for these analyses, we included only data from those studies where KPC was the predominant enzyme (>98%) in the analysis. We excluded methodologies that were not available commercially, except for the CDC protocol. Model-estimated sensitivities, specificities, and DORs with their corresponding 95% confidence intervals are shown in Table 7. Forest plots for the individual and aggregate studies are shown in Fig. 4 and 5.

Analysis of the results of the screening media in pure cultures shows that chromID ESBL, chromID Carba, and Supercarba have similar sensitivities. The results with Brilliance CRE medium and the CDC method with ertapenem overlap the 95% confidence intervals of chromID Carba and chromID ESBL media. Large confidence intervals can be seen with the CDC method, Colorex KPC, and CHROMagar KPC, reflecting the low number of tested isolates and conflicting results. For instance, CHROMagar KPC performed well in some studies (151, 158, 159) but not all studies (149, 155, 156), with sensitivities ranging from 40 to 98%.

Analysis of specificity is more homogeneous among the different methods. There is, however, a tendency for superiority favoring chromID Carba, while the opposite holds for chromID ESBL. This is expected, as the growth of ESBL-producing *Enterobacteriaceae* is considered a false-positive result when screening for CPE. Supercarba had a wide range of specificities, ranging from 35% to 82% depending on the details of the analysis, which is reflected in its large confidence interval. In this analysis, chromID Carba and Supercarba have a clear advantage in the clinic compared to the other methods. Given the large confidence intervals, these results must be interpreted with caution. Not included in the above-described analysis are data from a study by Hu et al., as it involved only 18 isolates of KPC-producing *Enterobacteriaceae* (105).

Analyses of medium performance on rectal/perirectal swabs are limited to those studies where KPC was the prevailing enzyme. Unfortunately, there are not enough data for a meaningful comparison of these media under different conditions. Available data with note of the enzyme distribution can be found in Table 6. The sensitivities for detection of KPC in rectal/perirectal swabs show overlapping confidence intervals for all methods, except for the CDC protocol, which is clearly inferior. Specificities also show significant overlap. HardyChrom agar showed the worst specificity, although it had a very large confidence interval, a product of

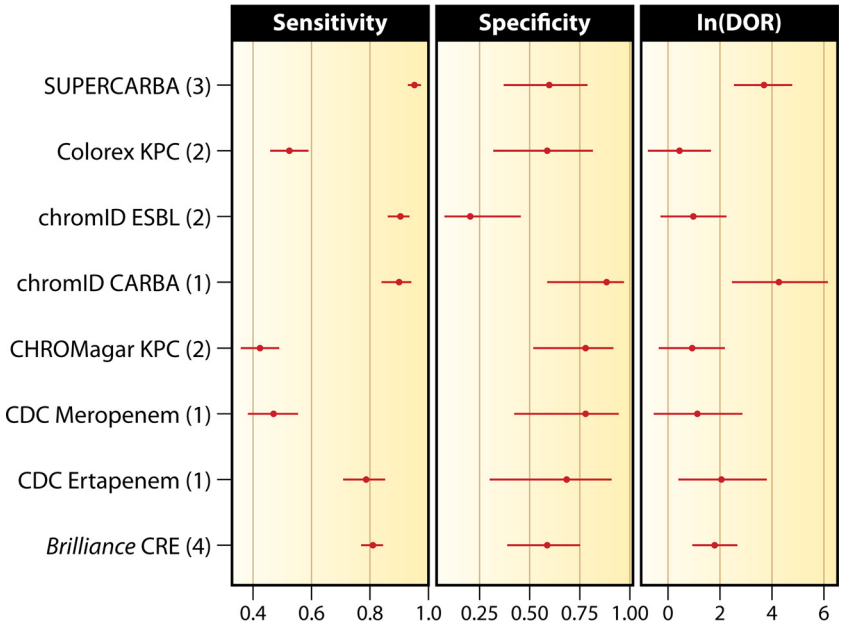

FIG 3 Aggregate estimates of sensitivity, specificity, and DOR for screening methods used on pure cultures. The number of studies used to calculate the performance of each method is shown in parentheses.

being tested in only one study (141). MacConkey agar with imipenem also performed acceptably in some studies (80, 111, 139, 150), showing sensitivities and specificities as high as 92% and 100%, respectively. Analysis of DORs shows homogeneity for most methods. The overall trend is for the CDC method to be inferior to the others. Improved performance is suggested for SpectraCRE, HardyChrom, and chromID Carba. The confidence intervals for HardyChrom and SpectraCRE, however, are exceedingly large. SpectraCRE was tested in a single study in a Chicago LTCF (156), which likely explains its large confidence interval.

Due to its limited scope, chromID OXA-48 agar was not included in this statistical analysis. In a study by Zarakolu et al., it showed 75% sensitivity when tested against clinical specimens containing OXA-48, with 99.3% specificity. When used in conjunction with chromID Carba, the sensitivity and specificity reached 90.9% and 98.5%, respectively (165).

The overall differences in sensitivity between the media can be explained by the carbapenemase being tested. Most media perform reasonably well with class A enzymes, while performance with class B and D enzymes is more variable. chromID Carba medium performed well both in pure culture and when tested on rectal/perirectal swabs. The Supercarba medium performed well on pure cultures. However, it was not tested with patient specimens. SpectraCRE performed well on rectal/perirectal swabs, although one must be aware of its confidence interval. The CDC method underperformed when tested on pure cultures and clinical specimens. Other methods that were tested, particularly those involving "house-grown" techniques, could not be analyzed with the same rigor, and unless more studies are done, we caution against their use in clinical practice.

It must be emphasized that many of the studies of these selective plates are limited to KPC-producing isolates. Furthermore, the various media evaluated in Tables 4, 6, and 8 are not available in all countries. Therefore, the practical issues of cost and availability affect the choice made by an individual laboratory, which must decide if optimal sensitivity is desired, knowing that additional workup will be required to detect false-positive results if the method has a low specificity.

Studies analyzing the LOD include bacteria with specific genetic backgrounds on pure cultures that may not necessarily represent the backgrounds present in a specific clinical setting (Table 8). The LOD will directly impact the sensitivity of the

TABLE 5 Comparison of model estimates of diagnostic performance for different screening methods using pure cultures[a]

Method (no. of studies)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	DOR (95% CI)	Aggregate no. of isolates (no. of positive isolates)
Brilliance CRE (4)	81.3 (77.1–84.88)	58.89 (39.31–76.02)	6.23 (2.6–14.91)	774 (439)
CDC protocol for ertapenem (1)	79.23 (71.21–85.47)	68.57 (30.95–91.4)	8.32 (1.49–46.57)	200 (130)
CDC protocol for meropenem (1)	46.92 (38.24–55.8)	78.57 (42.41–94.81)	3.24 (0.58–18.19)	200 (130)
CHROMagar KPC (2)	42.11 (35.69–48.81)	78.13 (52.18–92.12)	2.6 (0.73–9.27)	318 (228)
chromID Carba (1)	90.77 (84.36–94.72)	88.57 (59.31–97.63)	76.21 (11.97–485.11)	200 (130)
chromID ESBL (2)	91.01 (86.26–94.23)	21.27 (7.95–45.8)	2.74 (0.75–9.95)	376 (244)
Colorex KPC (2)	52.45 (45.78–59.04)	59.13 (32.06–81.6)	1.6 (0.48–5.35)	377 (230)
Supercarba (3)	96.27 (93.53–97.87)	60.38 (37.33–79.59)	39.29 (12.52–123.33)	176 (114)

[a] CI, confidence interval; DOR, diagnostic odds ratio.

screening method. Given the abundance of *Enterobacteriaceae* in stool, it is desirable to inhibit the growth of the carbapenem-susceptible population. However, this inhibition comes at the expense of sensitivity. A relatively low inoculum of a CPE isolate with borderline susceptibility will need to overcome this inhibitor, and the medium would have a higher LOD. On the other hand, adjusting growth inhibitors to obtain a lower LOD would allow for the growth of other bacteria and would decrease specificity.

High-resource settings where health care is already expensive may have a lesser impact on the isolation of more patients and may want to err on the side of higher sensitivities. Furthermore, the medical care provided in high-resource settings tends to be more invasive; therefore, there is a higher cost of missing a colonized patient. On the other hand, lower-resource settings may still benefit from the selection of a method with lower sensitivity that would decrease isolation costs while still having an impact on the local spread of CPOs.

Table 8 summarizes the limits of detection of the different agar screening media. All of the tested media and Supercarba agar performed reasonably well for the detection of class A enzymes (KPC), achieving a LOD in the range of 1×10^1 to 1×10^2 CFU/ml (80, 140, 143, 149, 166), except for chromID OXA-48, which, as expected, performs better with class D enzymes (OXA-48) (167). The LOD for class B enzymes on chromogenic media are ~1 log higher than those for class A enzymes (80, 140, 143, 149). MacConkey agar with disks had a LOD ~1 to 2 logs higher than those of the other media for both class A and B enzymes (80, 140). chromID OXA-48 showed poor performance for both class A and B enzymes, with a LOD of 1×10^7 CFU/ml (167). Class D enzymes were not tested by all methods. The LOD for Supercarba remained consistently close to 1×10^1 CFU/ml. Other methods showed a significant increase in their LOD for class D enzymes. Remarkably, chromID KPC, CHROMagar KPC, the CDC method, and chromID Carba had a LOD up to 6 logs higher than those of more sensitive methods (140, 143, 149). As expected, chromID OXA-48 performed exceptionally well with class D enzymes, with a LOD of 5×10^1 CFU/ml (167).

Nucleic acid amplification technology. Nucleic acid amplification technology (NAAT) detects the presence of a specific gene or genes, in most cases limiting its usefulness to previously characterized determinants. Furthermore, newly emergent variants of previously characterized genes may not be reliably detected. Since various genes can encode different carbapenemases, a broad panel of tests is needed to detect all targets. Because it is not practical to detect every enzyme, these tests have been designed to cover the most common carbapenemases. A challenge for nucleic acid-based testing is DNA extraction from stool. Feces contain PCR-inhibiting substances, and poor results may be obtained due to excessive shearing of DNA (168). Despite these concerns, very good methods are available for extracting DNA from stool, and multiplex molecular assays are routinely performed on stool specimens for gastrointestinal pathogens. It is critical to note that detection of resistant determinants in pure cultures or in specimens where a single organism is expected (such as blood or urine) is significantly easier than detection of the same genes in a more complex specimen such as a stool swab. In addition, epidemiological data such as species information are lost in most assays.

There are several NAAT-based methodologies that may be employed to detect carbapenemase genes in bacterial isolates (169). Theoretically, all of them can be used to screen stool specimens. These methodologies include single- and multiplex endpoint PCRs, loop-mediated isothermal amplification (LAMP), single- and multiplex quantitative real-time PCR (qPCR), and microarrays. Next-generation sequencing (NGS) may be another option, although it is not readily available in most clinical laboratories at this time (169). NGS remains expensive due to high equipment acquisition costs and the need for significant computer processing power and data storage (170).

Regardless of the NAAT-based methodology selected by a laboratory, there are complex regulatory requirements that vary from region to region. Implementation of a laboratory-developed assay involves the determination of the test's performance characteristics. The burden of an involved development-and-validation process may be partially relieved by the use of commercial assays. U.S. Food and Drug Administration (FDA) regulations in the United States are evolving at this time and will likely result in an increased regulatory burden on laboratories in the future.

Endpoint PCR is useful when there is a large quantity of the target gene. Specificity cannot be ensured unless positive results are confirmed by DNA sequencing or hybridization with specific probes. With proper validation, a PCR method can be acceptable. The Hyplex Super Bug ID system (Amplex Biosystems GmbH, Giessen, Germany) for the detection of carbapenemases is based on a multiplex endpoint PCR followed by enzyme-linked immunosorbent assay (ELISA) hybridization (171). Although it has not been tested directly on stool specimens, this NAAT showed 98% sensitivity and specificity for VIM-producing CPE when used on DNA extracted from clinical specimens, including blood, urine, pus, and respiratory samples, from Greece (172). Another multiplex endpoint PCR was developed by Voets et al. and allows the detection of a wide range of resistance genes (173). Some of these multiplex assays were developed by independent laboratories and are not widely available to most clinical laboratories. However, there is great value in demonstrating that these comprehensive assays can be developed.

Microarrays consist of oligonucleotides bound to a solid surface. The target gene of the pathogen is then labeled and hybridized to the immobilized probe. This reaction is then measured with a scanner (169). Microarrays are difficult to standardize (169), and reports describing the use of microarrays to directly screen for β-lactamase genes in stool are not available. Most assays, however, can be used to confirm and characterize the β-lactamase gene from suspicious colonies of a screening culture. These tests have excellent sensitivity and specificity, as shown by a study with 149 previously characterized *Enterobacteriaceae* that were subjected to a commercial Check-Points microarray assay (Check-Points Health, Wageningen, Netherlands), which was found to have 100% sensitivity and specificity (174). Direct testing of blood cultures also showed 98% concordance between a microarray method and routine microbiological testing (175). The Verigene BC-GN test is a microarray-like detection system. It detects nine genus/species targets and six resistance determinants, including KPC, NDM, OXA, VIM, and IMP, without the need for prior PCR amplification (176). Future studies are needed to determine if microarrays will be used to screen perirectal or stool specimens directly, although this may be hampered by their high cost and the advent of next-generation sequencing.

Loop-mediated isothermal amplification (LAMP) is a modification of conventional PCR where several oligonucleotides that bind to the target gene are incubated at the same temperature with

TABLE 6 Performance of culture methods on rectal/perirectal swabs[a]

Method	No. of positive swabs/ total no. of swabs	Sensitivity (%)	Specificity (%)	Enzyme, distribution (%)	Reference standard(s)	Reference
MacConkey agar with IPM	33/139	84.9	94.3	KPC, 100	PCR for KPC	80
MacConkey agar with IPM/MEM/ETP disks		75.8	89.6			
CHROMagar KPC		84.9	88.7			
chromID Carba	86/177	96.5	91.2	KPC, 98; VIM, 1	qPCR for KPC or VIM, mHT, aminophenyl-boronic acid–meropenem, and EDTA-meropenem testing	139
MacConkey agar with IPM		89.5	31.9			
CDC protocol		98.8	80.2			
MacConkey agar with ETP disk	97/189	96.9	98.9	KPC, 61.9; VIM, 9.3; KPC + VIM, 26.8; OXA-48, 3	Colony PCR for KPC, VIM, OXA-48, IMP, and NDM; negative isolates confirmed with a mHT	111
MacConkey agar + MEM alone or MEM + PBA, MEM + EDTA, and MEM + PBA		94.8	100			
MacConkey agar with MEM	92/200	89.1	85.2	KPC, 68; VIM, 31	PCR for KPC, IMP, NDM, VIM, and OXA-48; Vitek susceptibility testing and mHT	140
CDC protocol		89.1	86.4			
Enriched brain heart (BH) culture with ertapenem replated onto chromID ESBL agar		92.4	93.3			
chromID Carba		92.4	96.9			
chromID ESBL		92.4	84.7			
chromID Carba	32/175[c]	100	98	NDM, 100	NDM PCR, disks with meropenem, boronic acid, cloxacillin, and dipicolinic acid (Rosco KPC/MBL Confirm kit)	157
Brilliance CRE		59	34		Agar dilution MICs for carbapenems, negative Rosco KPC/MBL test	
CDC protocol	33/149	65.6	49.6	KPC, 100	KPC PCR; broth microdilution susceptibilities for ertapenem, imipenem, and meropenem; mHT	150
MacConkey agar with ETP disk with a 27-mm breakpoint		97	90.5			
MacConkey agar with IPM, MEM, and ETP disks	41/122	92.7	95.9	KPC, 100	Direct KPC PCR on swab	151
CHROMagar KPC		100	98.4			
MacConkey agar with MEM and ETP disks	54/187[f]	87	100	KPC, 100	qPCR on swab and isolates followed by gel electrophoresis and sequencing	152
MacConkey agar with IPM	64/755	87.5	99.4	KPC, 100	KPC PCR, KPC PCR, mHT, repeat culture, repeat PCR	153
chromID Carba (prototype)	64/37,200[c]	100	93	NDM, 100	PCR for IMP, VIM, GIM, SPM, SIM, and NDM; mHT	158

Colorex KPC		97	96			
CHROMagar KPC	46/126	97.8	98.7	KPC, 72.5; VIM, 27.5	Phoenix susceptibility; EDTA/IMI confirmatory disks; PCR for KPC and VIM	159
MacConkey agar with IPM		78.3	97.5		Negative result not confirmed if negative by both methods	
HardyChrom	46/126	76.1	100[b]	KPC, 100	qPCR[e] for KPC and NDM; PCR for SME, VIM, IMP, GES, OXA-48, and AmpC	141
CDC protocol		78.3	100[b]			
Supercarba	10/77	80	98.5[b]	OXA-48, 100	For positive and negative results, PCR[e] for KPC, NDM, VIM, IMP, NDM, OXA-48, and ESBL panel	160
Supercarba with enrichment step		100				
chromID ESBL		90	68.6			
chromID ESBL with enrichment step		100				
Brilliance CRE		80	86.6			
Brilliance CRE with enrichment step		100				
CHROMagar KPC	66/95	77.3	100	KPC, 100	Positive results confirmed with PCR[e]; negative results had negative qPCR[e] and PCR results	155
VAN/AMB/CAZ/CLI plate		77.3	100			
CHROMagar KPC	47/150[d]	76	75.7	KPC (presumed)	Confirmed with KPC qPCR and Microscan susceptibility testing	156
SpectraCRE		97.8	86.4			
MacConkey agar with ETP disk		83	73.8			
CDC protocol	33/302	57.6	95.2	OXA-48, 100	Initial screen with inhibition disk synergy testing followed by PCR and sequencing of samples with positive results	165
chromID OXA-48		75.8	99.3			
chromID Carba		57.6	98.9			
chromID Carba + chromID OXA-48		75.8	94.4			

[a] Abbreviations: MEM, meropenem; IPM, imipenem; ETP, ertapenem; VAN, vancomycin; CLI, clindamycin; CAZ, ceftazidime; AMB, amphotericin B; mHT, modified Hodge test.
[b] Nonfermenting bacteria were excluded.
[c] Stool specimens.
[d] Perirectal swabs.
[e] PCR was done directly on the specimen. If not noted, PCR was performed on pure cultures derived from the sample.
[f] Includes both perianal and perirectal swabs.

TABLE 7 Comparison of model estimates of diagnostic performance for screening methods for detection of KPC-producing *Enterobacteriaceae* using rectal/perirectal swabs[a]

Method (no. of studies)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	DOR (95% CI)	Aggregate no. of tested swabs (no. of positive swabs)
CDC method (3)	85.37 (58.05–96.09)	82.94 (41.85–97.05)	28.37 (0.67–1,209.67)	452 (165)
CHROMagar KPC (4)	85.16 (61.39–95.4)	92.74 (70.56–98.55)	73.35 (1.96–2,743.26)	506 (187)
chromID Carba (1)	95.98 (65.92–99.66)	90.76 (30.51–99.55)	234.36 (4.99–10,996.66)	177 (86)
HardyChrom (1)	75.53 (22.42–97.06)	99.38 (72.54–99.99)	497 (4.9–50,431.85)	126 (46)
SpectraCRE (1)	96.88 (65.46–99.8)	86.06 (22.15–99.26)	191.34 (3.45–10,605.72)	150 (47)

[a] CI, confidence interval; DOR, diagnostic odds ratio.

the DNA polymerase. As DNA polymerizes, there is a release of pyrophosphate that can be detected with a fluorescent dye or a compound that will increase the turbidity of the solution (177). This method's advantages include increased sensitivity with low DNA concentrations compared to endpoint PCR, no need for a thermocycler, and simple visualization of the result. LAMP assays can be particularly useful in low-resource settings (169). A LAMP assay for the detection of NDM-1 was successfully used on 336 clinical specimens, including rectal swabs (178). Those investigators found a limit of detection of 10.70 pg/μl of genomic DNA, which would correspond to roughly 1×10^3 CFU, compared to 1,070 pg/μl (or 1×10^5 CFU) for the endpoint PCR assay used as a comparator in that study. Solanki et al. developed two LAMP assays for the detection of KPC and NDM-1 (179). These assays were able to detect all 48 tested isolates with either NDM or KPC, while endpoint PCR detected only 44. Other studies have found an improved performance of LAMP versus endpoint PCR for microbiological targets other than CPE but not versus real-time assays (180, 181). Therefore, LAMP assays may have a useful role in detecting CPOs, but they are not the most sensitive assay for clinical microbiology laboratories that have access to other types of NAATs.

Real-time or quantitative PCR (qPCR) is based on the coupling of PCR with the detection of the amplified target. Real-time PCR has been used for the screening of CPO using both commercial and "in-house" kits, with the advantage of more rapid results, increased sensitivity, and increased specificity (152, 153, 182, 183). A recent seven-center study in the Netherlands found 100% sensitivity and specificity for a multiplex assay used for the detection of KPC, NDM, VIM, IMP, and OXA-48 in 20 selected laboratory isolates (184).

Many laboratories have experience in using qPCR for the direct screening of stool/rectal swab specimens. Examples of stool/rectal swab testing with qPCR in routine clinical practice include screening for vancomycin-resistant enterococci, group B streptococci, and *Clostridium difficile* (185–187). The validity of the use of qPCR, including for quantification of KPC carriage loads, was evaluated by Lerner et al. (188). They determined a detection limit of 10 plasmid copies, which we presume is close to 1×10^1 CFU/ml.

Another in-house qPCR for NDM-1 found a limit of detection of 1×10^1 to 3×10^1 CFU/ml of stool, compared to 2×10^1 to 1×10^2 CFU/ml for chromID ESBL and 2×10^1 to 4×10^3 CFU/ml for CHROMagar KPC (189). An additional study found limits of detection with endpoint PCR of 1×10^4 to 1×10^5 CFU/ml for KPC and 1×10^3 CFU/ml for NDM (141). Using an in-house qPCR assay, Naas et al. found a limit of detection for OXA-48 of 1×10^1 to 1×10^2 CFU/ml in stool for qPCR, compared to 1×10^1 to 1×10^2 CFU/ml for Supercarba and 2×10^1 to 3×10^2 CFU/ml for chromID ESBL (182). A comparison between agar screening and qPCR for KPC showed 100% sensitivity for the qPCR assay, compared to 77% for culture methods (155). Overall, the limits of

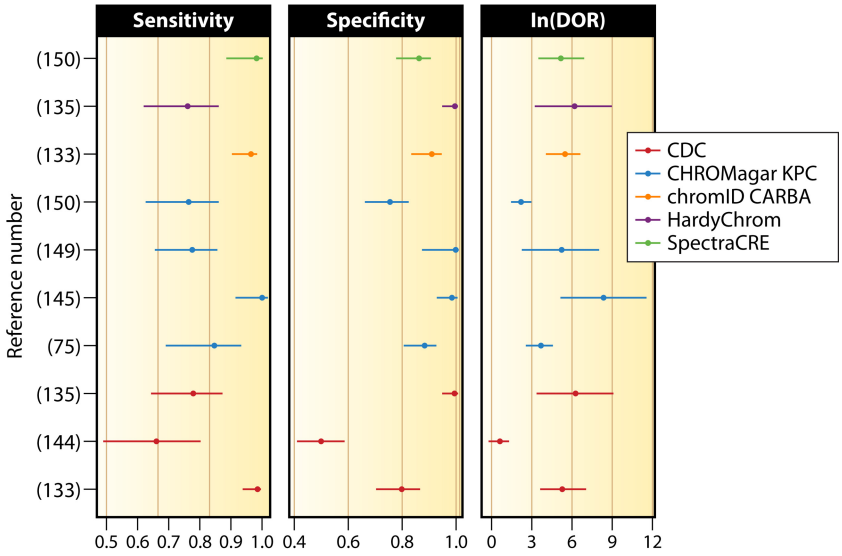

FIG 4 Per-observation estimates of sensitivity, specificity, and DOR for screening of rectal/perirectal swabs (75, 133, 135, 144, 145, 149, 150).

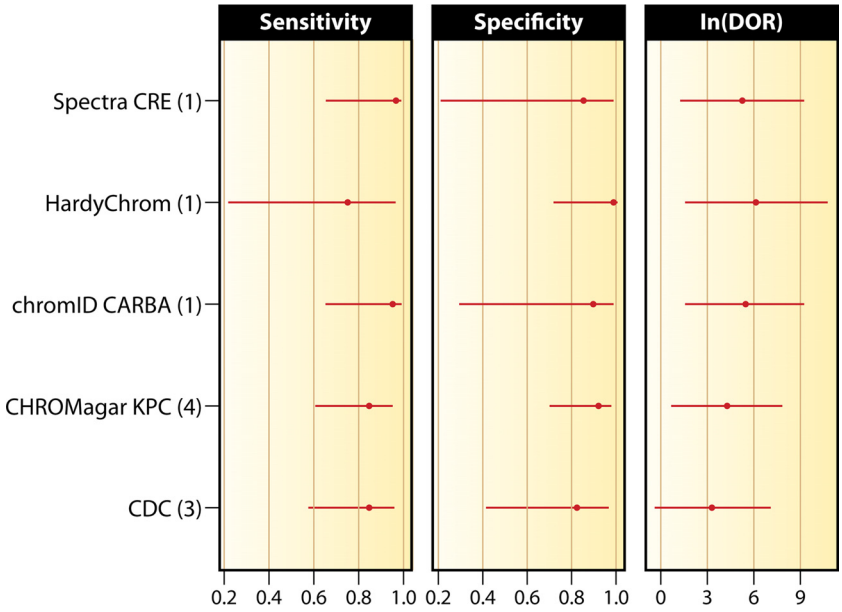

FIG 5 Aggregate estimates of sensitivity, specificity, and DOR for screening methods using rectal/perirectal swabs. The number of studies used to calculate the performance of each method is shown in parentheses.

detection for single-gene assays (152, 155, 182, 190) tend to be lower than those for multiplex assays (141, 191).

Commercial assays for molecular multiplex CPO detection include Check-Direct CPE, Check-MDR Real Time (Check-Points Health), Hyplex SuperBug ID (Amplex Biosystems), Eazyplex SuperBug CRE (Amplex Biosystems), and Xpert MDRO (Cepheid). Check-MDR Real Time consists of an oligonucleotide probe that binds to the target sequence (VIM, NDM, KPC, and OXA-48), to a pair of universal primers, and to a molecular beacon. Real-time PCR amplifies only the bound target sequences at the same time

TABLE 8 Geometric mean limits of detection in pure cultures according to culture method and β-lactamase class[a]

Method	Geometric mean limit of detection (CFU/ml)			No. of isolates of class A/B/D	Reference
	Class A	Class B	Class D		
Supercarba	1.47×10^1	3.36×10^1	1.37×10^1	18/52/44	149
chromID ESBL	1×10^1	1.26×10^1	1.21×10^3	18/52/44	
chromID KPC	8.1×10^1	1.64×10^3	3.43×10^6	18/52/44	
Supercarba	1.41×10^1	2.81×10^1	1.59×10^1	20/51/43	143
Brilliance CRE	1.12×10^2	2.86×10^2	1.45×10^3	20/51/43	
CHROMagar KPC	5.89×10^1	8.93×10^2	4.62×10^6	20/51/43	
MacConkey agar with imipenem	4.68×10^2	1.24×10^3	NT	8/2/0	80
MacConkey agar with meropenem/ertapenem disks	2.62×10^6	3.32×10^5	NT	8/2/0	
CHROMagar KPC	2.02×10^3	1.24×10^4	NT	8/2/0	
CDC method	6.87×10^1	8.66×10^2	5.2×10^7	5/2/1	140
chromID ESBL with prior enrichment in BHI broth + 10 μg ertapenem	2.6×10^1	5.55×10^1	ND	5/2/1	
chromID ESBL	7.49×10^1	4.42×10^2	ND	5/2/1	
chromID Carba	2.11×10^1	4.42×10^2	5.5×10^7	5/2/1	
Brilliance CRE	2.67×10^1	3.41×10^1	3.77×10^1	12/14/5	166
chromID OXA-48	1×10^7	ND	3.36×10^1	10/10/57	167
chromID Carba	1×10^1	2.0×10^1	1.62×10^4	10/10/57	
Supercarba	3.16×10^1	2.51×10^2	2.98×10^1	10/10/57	
CHROMagar KPC	ND	ND	1.26×10^4	0/0/9	100
CHROMagar ESBL	ND	ND	5.26×10^3	0/0/9	

[a] NT, not tested; ND, not detected; BHI, brain heart infusion.

TABLE 9 PCR-based testing for CPOs in clinical specimens

Target(s)	Methodology	Specimen type(s)[b]	Sensitivity (%)	Specificity (%)	Limit(s) of detection (CFU/ml)	No. of specimens	Reference
KPC	PCR	ES	92.2	99.6	Not calculated	755	153
KPC	qPCR	ES	97	96.6	4×10^0–4×10^2	95	155
KPC	qPCR	S, MC	100	98	5×10^0 CFU/ml	216	190
VIM, IMP, KPC, OXA-48, NDM-1	PCR (Hyplex SuperBug ID)	MC	98.0	98.6	Not calculated	236	172
OXA-48	qPCR	PC, SS	100	100	10^1–10^2	35	182
KPC, NDM-1	qPCR	S, SS	100	100	KPC, 10^4–10^5; NDM, 10^3	46/80[a]	141
KPC	qPCR (EasyQ KPC)	S	93.3	99	Not calculated	806	193
VIM, OXA-48, NDM, KPC	qPCR (Check-MDR Carba)	SS	100	100	10^3–10^5	25	191
KPC, NDM, VIM	qPCR (Xpert MDRO)	S, SS	100	99	$<3 \times 10^2$	328	183
KPC	qPCR	S	97.9–100	95–96.4	5×10^0	187	152
NDM-1	qPCR	SS	100	Not calculated	10^1–3×10^1	32	189

[a] Specificity panel of 80 known negative specimens.
[b] ES, stool or perirectal swab with prior enrichment; S: stool, stool swabs, or perirectal swabs; MC, mixed clinical specimens; SS, spiked stool or stool swabs; PC, pure cultures.

that the molecular beacon emits fluorescence to measure amplification. The manufacturer has established a limit of detection of <5 copies per reaction. Testing of pure cultures showed 100% sensitivity and specificity (192). The manufacturer, however, recommends the use of pure cultures, which is clearly not the method used to screen perirectal swabs directly. Check-Direct CPE is a real-time assay using probe detection chemistry. It has a limit of detection of 5 copies per reaction. Using "spiked" stool specimens, Check-Direct CPE was able to detect a bacterial inoculum of 10^3 to 10^5 CFU/ml, with less sensitivity for KPC (191). NucliSENS EasyQ KPC (bioMérieux) is another real-time assay that uses molecular probes. This assay was compared to chromID ESBL with ertapenem disks by using surveillance specimens. Although a limit of detection was not determined, the assay showed 93% sensitivity (193). The SuperBug CRE system is a multiplex LAMP system that is able to detect KPC, VIM, NDM, and OXA-48 (and some variants) in addition to the ESBLs CTX-M-1 and CTX-M-9. On pure cultures, Eazyplex SuperBug CRE was able to correctly identify all 139 *Enterobacteriaceae* isolates (194). However, when used against a panel of 82 *Acinetobacter* species isolates, it produced 5 false-positive results (195). The Xpert MDRO assay has been used to detect CPO directly from rectal and perirectal swabs. The assay was able to detect KPC, NDM, and VIM with 100% sensitivity and 99% specificity using 328 discarded perirectal, rectal, or stool samples from two U.S. hospitals and one Spanish hospital (183).

Table 9 summarizes the molecular methods that have been used on clinical specimens. Some of the reports show excellent sensitivity and specificity for molecular assays; however, other studies show a broad range for the LOD of specific carbapenemases, with numbers comparable to those for agar-based methods. Limitations include the cost and the inability to detect new or unanticipated carbapenemases. Pooling of specimens for initial screening, followed by confirmatory testing of positive samples, may be an option to contain costs, especially in low-prevalence settings, but investigations are needed to see if the loss of sensitivity is too great. The cost-effectiveness of the approach will also depend on the prevalence of CPOs at an individual institution, which will determine the number of specimens that would require follow-up testing when a positive pooled result is obtained, because all individual specimens in the pool would need to be retested. The concern about detecting new, not-yet-described, carbapenemases will need to be addressed by constant vigilance in updating targets in a chosen assay; if a laboratory reports a new carbapenemase in the local geographic region, or when a medical center treats a large volume of international patients, adjustments will need to be made.

SCREENING OPTIONS

As discussed above, there are several screening options that may be easy to implement in a clinical microbiology laboratory. Any of these options should be closely coordinated with the infection control program of the institution. One must know the baseline prevalence and type of resistance enzymes in a specific setting, as the choice of method will be dependent on these variables. At this time, we cannot find data to suggest any advantage of using stool specimens versus rectal or perianal swabs. Most studies have been done using rectal swabs, and it is likely that most institutions would tend to prefer this modality.

Whatever modality is chosen for screening, laboratories must be aware that local validation will be required. When implementing a screening program, it is important to consider certain factors (Table 10). The decision regarding who should be screened will always be controversial, as the balance of cost and risk will be subject to different interpretations. Ideally, screening should be universal, but most institutions do not have the necessary resources for both screening and isolation while waiting for test results. Table 11 proposes a set of criteria that can be used to screen certain patient populations.

Culture-Based Screening with Molecular Confirmation

Culture-based screening includes the use of a chromogenic agar, Supercarba, or the CDC method to perform perirectal or rectal swab screening of patients. While universal screening has a higher potential for detection and prevention of outbreaks, it comes at a significant financial cost. Based on our analysis, we favor the use of chromID Carba. However, if the hospital is located in a geographic area with a high incidence of OXA-48, the clinical microbiology laboratory should strongly consider the use of Supercarba or the addition of an OXA-48-specific medium such as chromID OXA-48 medium. A biplate containing chromID Carba and chro-

TABLE 10 Factors to consider when implementing a screening program

Factor
Epidemiology of CPO in the community
Prevalence of each carbapenemase
Ability to identify high-risk groups
Availability and cost of isolation beds
Existing logistics for collecting specimens (e.g., an existing screening program for carriers of vancomycin-resistant *Enterococcus* spp.)
Current clinical microbiology laboratory capabilities
Availability of molecular diagnostic tools
Available technologists and ability to accommodate testing
Experience developing and validating in-house assays
Experience/availability of other technologies to detect carbapenemases
Carba NP or Blue-Carba
Inhibition disk assays (double-disk synergy test)
UV spectrometry or MALDI-TOF MS
Modified Hodge test

mID OXA-48 is available (chromID Carba Smart; bioMérieux, France). Bacteria that grow on Supercarba medium should be identified by conventional microbiological tests or MALDI-TOF MS. Similarly, isolates on chromogenic media that cannot be readily classified as *Enterobacteriaceae* should also be identified. While non-*Enterobacteriaceae* can carry plasmids encoding carbapenemases, commonly, carbapenem resistance in these organisms is mediated by other mechanisms. The decision to isolate patients with carbapenem-resistant organisms other than *Enterobacteriaceae* should be based on local epidemiology. Confirmation of positive specimens should ideally be sought with molecular testing with either a broad panel of PCRs or a microarray method. Alternatively, a phenotypic test (such as Carba NP, Blue-Carba, inhibitory disk synergy testing, or mHT) can be used to confirm the presence of carbapenemases, reserving molecular testing for a random sample of positive isolates. Random sampling will come at the cost of decreased hospital epidemiology data. To track the prevalence and type of carbapenemases in an institution, isolates from clinical specimens, not only surveillance specimens, demonstrating decreased carbapenem susceptibility should also be subjected to an assay for the detection of carbapenemase activity or a PCR panel/microarray.

Figure 6 proposes an algorithm for the use of a culture-based approach. Note that depending on the laboratory capabilities, some samples may be referred to a research laboratory.

Molecular-Based Approach

The use of universal screening of perirectal swab samples via molecular methods may not be desirable or affordable due to low prevalence or increased costs. Screening of high-risk patients, such as those returning from areas of endemicity, those who have been transferred from LTCFs, or those who have had extensive exposure to carbapenems (41), may be advisable. A multiplex real-time PCR assay that includes KPC, OXA-48, NDM, and VIM should be used in most locales. However, laboratories in specific areas where IMP or GES-5 is common should either develop their own assays or perform simultaneous routine culture testing. Specimens with indeterminate results and a sample of negative specimens obtained through molecular testing should be tested with a culture-based method with high sensitivity, such as Supercarba or chromID Carba. Suspect colonies should be subjected to antimicrobial susceptibility testing or to a test for carbapenemase activity (e.g., Carba NP or Blue-Carba). A test such as the double-disk synergy test with avibactam-ertapenem with follow-up testing with ertapenem-boronate or moxalactam-EDTA, depending on the result, may enable a laboratory to distinguish between class A, B, and D enzymes (113), making it particularly attractive for this scenario. Figure 7 suggests an algorithm for CPO screening based on molecular methods.

Combined Approaches

Some combined approaches can be useful in specific situations, such as during an outbreak caused by a CPO with a known enzyme. We speculate that a LAMP or qPCR assay could be implemented for universal screening of carbapenemases involved in an outbreak. Patients who test positive could be quickly cohorted, while patients who test negative could be subjected to routine culture-based screening. This strategy would maximize the number of available hospital beds while attempting to minimize the number of patients under enhanced infection control precautions.

CONCLUSIONS

In this review, we stress that screening for intestinal carriage of CPOs is of significant importance for the development of infection control strategies. However, the optimal screening modality remains to be established for each location and for each specific purpose. Culture-based screening methods have the advantage that they involve technologies that are readily available in clinical microbiology laboratories. Some enhancements, such as the use of

TABLE 11 Proposed criteria for screening of patients for CPO infection

Area	Patients for screening
Areas where the disease is not endemic	Patients with multiple hospital admissions ICU patients Patients who have received medical care in areas of endemicity over the last 12 months Patients who reside in health care settings Patients with history of CPO infections or colonization Patients with prior prolonged hospital stays Patients coming from areas of endemicity Patients who are or who are expected to become incontinent or unable to take care of their personal hygiene
Areas where the disease is endemic	Everyone (as resources for testing, isolation, and cohorting allow), with particular emphasis on critically ill patients, patients who are unable to take care of their excreta, and patients with an expected prolonged hospital stay

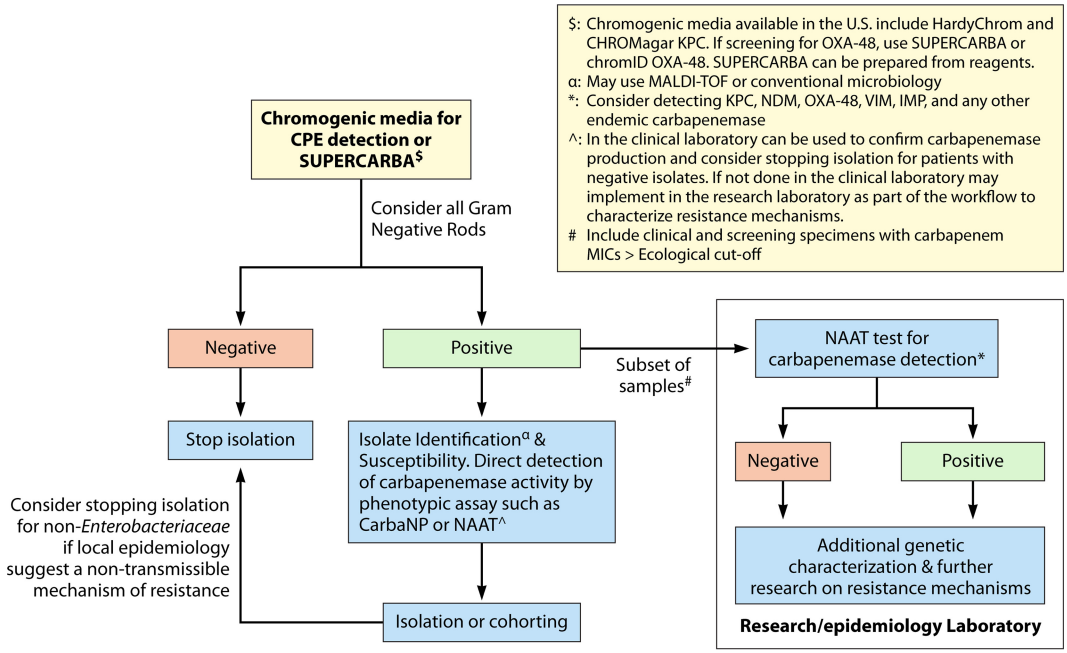

FIG 6 Screening by conventional microbiology.

chromogenic media, make culture-based screening more convenient; however, the turnaround time is long, and the sensitivity of some culture methods is not as high as desired. In addition, culture-based methods may not be optimal for the detection of low-level carbapenemase production, which is important for epidemiological purposes (93).

Agar-based procedures always require confirmatory testing to detect the type of *bla* gene present after a potentially resistant isolate is detected. Clinical microbiology laboratories may choose an agar-based screen with follow-up molecular testing or a molecular method with reflex to culture if further investigation of the isolate is desired. On the other hand, NAATs offer a promising approach for screening for carriage of CPOs. These methods offer faster availability of results and increased sensitivity but with a significantly increased expense and an unclear specificity with direct specimens at this time.

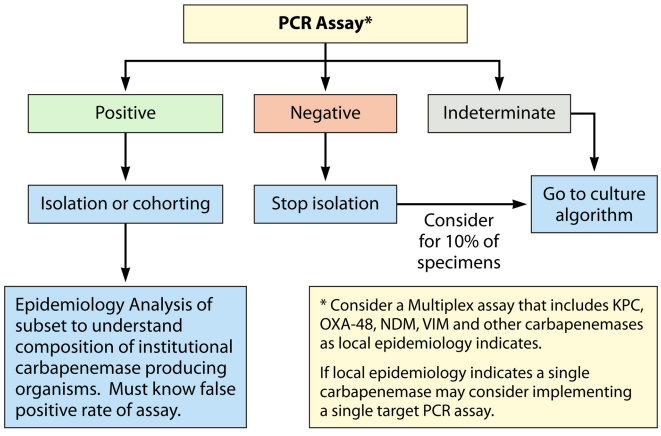

FIG 7 Molecular screening algorithm.

Our review indicates that chromID Carba and Supercarba media have excellent sensitivities for class A β-lactamases that rival that of real-time PCR. As there are more communities where KPC is becoming endemic, the use of screening for this class of enzymes may become less useful because the high prevalence could make empirical therapy and initial isolation procedures prior to surveillance results default to the assumption of a KPC-positive isolate. It is still hoped, however, that communities in which KPC-positive organisms are not endemic may contain the spread of resistant isolates for some time. We argue that screening should shift to those carbapenemases that are threatening to become common and that have a high potential of causing outbreaks, such as NDM and OXA-48. Real-time PCR appears to be ideally suited for this goal; however, qPCR implementation is hampered by cost. In addition, there could be false-positive results for OXA-48 due to the amplification of similar chromosomally encoded enzymes in species such as *A. baumannii*. Furthermore, data on their performance in the clinical setting compared to that of culture-based screening are not yet available. Nonetheless, the improved turnaround time and improved accuracy of NAATs and direct carbapenemase detection assays may result in limiting the unnecessary prolonged isolation of newly admitted patients, thus decreasing costs to the infection control program. Nevertheless, molecular tests with high sensitivity have a cost that is difficult to offset and can be prohibitive for many clinical microbiology laboratories (136). We must choose wisely.

There is an urgent need to define the appropriate criteria and clinical circumstances to conduct screening for gastrointestinal carriage of CPOs and to determine the optimal methods to be used. The best method will differ from institution to institution depending upon the prevalence in the community, the travel patterns and demographics of the population, the level of care rendered by the hospital, the age of patients, the

technical capabilities of the microbiology laboratory, and the resources allocated by the hospital administration for infection control monitoring.

Agar-based and colorimetric screens are usually more affordable and are able to detect the presence of "new and emerging" carbapenemases before they are characterized. In addition, most agar-based tests can be performed without the need for significant investments by the clinical microbiology laboratory. However, they may lack sensitivity for carbapenemase producers that confer a low level of resistance. Furthermore, variability in performance according to the β-lactamase class makes the selection of a particular method more difficult. For instance, it is easier to justify the resources needed to perform a screening test that will reliably detect class A carbapenemases in an area where KPC is endemic. At the same time, the detection of class D carbapenemase producers may allow for the institution of a program that will prevent them from becoming prevalent. Laboratories may consider a combined approach of two independent assays to screen for a broader spectrum of carbapenemases. This strategy, however, comes with the disadvantage of increased cost and labor.

At this time, clinical microbiology laboratories that choose to implement a CPO screening methodology must have a reliable procedure for the detection of the carbapenemases in their area of endemicity. In most of the United States, this carbapenemase would be KPC, while laboratories in Europe and the Middle East should likely screen for OXA-48. Once a *bla* gene is found, laboratories should choose which of the other carbapenemases that they want to include in their screening approach, knowing that they will miss colonized patients carrying a CPO not included in their infection control algorithm.

Although this review focuses on carbapenemase-producing *Enterobacteriaceae*, plasmids carrying carbapenemase genes have been identified in non-*Enterobacteriaceae*, so vigilant monitoring of both types of organisms may be warranted in the future. We assert that until a carbapenem-resistant isolate is recovered in a clinical culture and detected by routine susceptibility testing, the number of carbapenemase targets to be included will be determined by cost, time available for labor, and technical abilities of the laboratory. It is likely easier to adapt an in-house molecular assay than a commercial assay to detect emerging carbapenemases by adding additional primers and probes to an existing assay, which provides information about both the presence of enzymes and the type of enzyme. In contrast, commercially available assays have the advantage of manufacturer validation, but they cannot be modified quickly and are modified only when the manufacturer chooses to update the assay. Continued surveillance to detect carbapenemases not detected with the chosen assay is warranted. These considerations are most important to prevent and control infections caused by carbapenemase producers and protect public health. A proactive approach to halt the spread of carbapenemase producers is desperately needed.

ACKNOWLEDGMENTS

Research reported in this publication was supported in part by the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH) under award numbers R01AI100560, R01AI063517, and R01AI072219 to R.A.B.; NIAID DMID protocol 10-0065 to K.K.; the Intramural Research Program of the NIH, the Cleveland Department of Veterans Affairs, Veterans Affairs Merit Review Program Award 1I01BX001974, and Geriatric Research Education and Clinical Center VISN 10 to R.A.B.; and the Swiss National Science Foundation under grant number 32003B_153377 to A.E.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

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