Rapid and Direct Real-Time Detection of bla_{KPC} and bla_{NDM} from Surveillance Samples

Shawn Vasoo,[a] Scott A. Cunningham,[a] Peggy C. Kohner,[a] Jayawant N. Mandrekar,[b] Karen Lolans,[c] Mary K. Hayden,[c,d] Robin Patel[a,e]

Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology[a] and Department of Health Sciences Research,[b] Mayo Clinic, Rochester, Minnesota, USA; Department of Pathology, Rush University Medical Center, Chicago, Illinois, USA[c]; Section of Infectious Diseases, Rush University Medical Center, Chicago, Illinois, USA[d]; Division of Infectious Diseases, Department of Internal Medicine, Mayo Clinic, Rochester, Minnesota, USA[e]

We assessed the performance of a duplex real-time PCR assay for bla_{KPC} and bla_{NDM} performed directly (D-PCR) on perianal and perirectal swabs and stool. Spiked specimens and 126 clinical surveillance swabs (comprising a sensitivity panel of 46 perirectal double swabs previously determined to be culture positive for bla_{KPC}-PCR-positive Enterobacteriaceae and a specificity panel of 80 perianal swabs from patients at risk of carbapenemase-producing Enterobacteriaceae [CPE] colonization) were studied. For the surveillance swabs, D-PCR was compared to PCR after broth enrichment (BE-PCR) and two culture-based methods: the HardyCHROM ESBL agar (HC-A) and the CDC screening (CDC-A) methods. PCR was performed on morphologically distinct colonies that were isolated by culture. All of the initial PCR testing was done without extraction using a simple lysis procedure. The analytical sensitivities of D-PCR for bla_{KPC} were 9 CFU/μl (for swabs) and 90 CFU/μl (for stool), and for bla_{NDM}, it was 1.9 CFU/μl (for both swabs and stool). In the clinical sensitivity panel, D-PCR and BE-PCR were initially positive for bla_{KPC} in 41/46 (89.1%) and 43/46 (93.5%) swabs, respectively. The swabs that were initially negative by D-PCR ($n = 5$) and BE-PCR ($n = 3$) were visibly stool soiled; all swabs were bla_{KPC} positive upon repeat testing after lysate extraction. The CDC-A and HC-A yielded bla_{KPC}-positive Enterobacteriaceae from 36/46 (78.3%) and 35/46 (76.1%) swabs, respectively (sensitivities of D-PCR/BE-PCR postextraction of soiled specimens versus HC-A, $P = 0.0009$, and versus CDC-A, $P = 0.0016$). All swabs in the specificity panel were negative for CPE by all four methods. D-PCR allows for the timely detection of bla_{KPC} and bla_{NDM} carriage with excellent sensitivity when specimens visibly soiled with stool undergo preparatory extraction.

Active surveillance for carbapenemase-producing Enterobacteriaceae (CPE) is a component of successful outbreak control (1–3). The recently updated Centers for Disease Control and Prevention (CDC) recommendations include screening hospitalized patients who are epidemiologically linked contacts of patients who carry CPE or who have had a history of hospitalization overseas in the last 6 months (4). Klebsiella pneumoniae carbapenemase (bla_{KPC}) and New Delhi metallo-β-lactamases (bla_{NDM}) are the carbapenemases of foremost current concern in the United States.

Various culture-based methods utilizing rectal and perianal swabs or stool have been used for the detection of CPE colonization. These include several commercially available chromogenic media (5–7), none of which are currently cleared by the FDA, and laboratory-developed methods which utilize selective enteric media containing carbapenems (6) or onto which carbapenem disks are placed (6, 8, 9). The CDC-recommended culture method entails overnight selective broth enrichment culture of surveillance swabs, followed by plate culture and testing of suspect colonies using the modified Hodge test (MHT) or formal antimicrobial susceptibility testing (10). Culture methods, while yielding organisms for further characterization, require an incubation period of at least overnight and can take days. Also, while culture methods may yield carbapenem-resistant Gram-negative bacilli (CR-GNB), these may not necessarily be carbapenemase-producing organisms, as carbapenem resistance mediated by other mechanisms (e.g., AmpC β-lactamases or extended-spectrum β-lactamases with porin loss) may result in carbapenem nonsusceptibility, diminishing the specificity of culture-based screening tests for the detection of CPE (7). The MHT itself lacks both sensitivity (especially for the metallo-β-lactamases) and specificity (11).

The importance of accurate and rapid characterization of CPE for infection control was illustrated recently during an outbreak of bla_{KPC}-positive K. pneumoniae at the National Institutes of Health Clinical Center, in which prompt laboratory response and infection control measures, including the implementation of molecular testing directly from swabs, mitigated the outbreak (12, 13).

Several laboratory-developed PCR-based nucleic acid amplification tests (NAATs) have been described for the detection of carbapenemases in surveillance swabs and stool (8, 14–19). Commercial assays, including a nucleic acid sequence-based amplification (NASBA)-based assay, the NucliSENS EasyQ KPC assay (bioMérieux SA, Marcy l'Etoile, France), have also been evaluated on surveillance swabs (20, 21). A multiplex PCR assay (hyplex SuperBug ID and hyplex-MBL ID; Amplex Diagnostics GmbH, Gars-Bahnhof, Germany) has been developed for use directly on clinical material for the detection of carbapenemases (22, 23), although data on its performance on surveillance swabs are lacking. More recently, a multiplex PCR assay targeting Klebsiella pneumoniae carbapenemase (KPC), New Delhi metallo-β-lactamases (NDM), the OXA-48 group, and VIM, the Check-Direct CPE (Check-Points Health B.V., Wageningen, the Netherlands) was

developed for performance on surveillance swabs or cultured isolates (24), although there are no published data on this assay at the time of this writing.

Most of the abovementioned assays performed on surveillance swabs have been limited in one or more ways; most are single-plex assays (8, 14, 15, 18–21) targeting only bla_{KPC}; and some entail a broth enrichment step that delays the turnaround time by a day (15, 18, 19) or require a prior extraction step (8, 14, 20, 21, 24).

NAAT testing for the two most commonly encountered carbapenemases in the United States performed directly on surveillance swab specimens with minimal preparatory steps, preferably avoiding upfront nucleic acid extraction, should provide results that translate into timelier implementation of infection control measures. Recently, we described a duplex bla_{KPC} and bla_{NDM} assay for the direct testing of isolated colonies (25). In this study, we applied our assay to spiked surveillance specimens (perianal or perirectal swabs and stool). We also compared the performance of the duplex PCR assay done directly (D-PCR) on a set of clinical surveillance perianal or perirectal swabs after a simple lysis procedure and after a broth enrichment step, alongside two culture-based methods, the CDC-recommended method (CDC-A) (10), and a commercially available chromogenic medium, HardyCHROM ESBL agar (HC-A) (Hardy Diagnostics, Santa Maria, CA).

(This paper was presented in part at the 9th International Symposium of Antimicrobial Agents and Resistance, 13 to 15 March 2013, in Kuala Lumpur, Malaysia.)

MATERIALS AND METHODS

Limit of detection. The perianal swabs and stool used for the spiking studies were routine specimens submitted to our clinical laboratory for testing for other reasons and were shown to be negative for bla_{KPC} and bla_{NDM} by our assay. Serial 10-fold dilutions (10^{-1} to 10^{-8}) of two reference isolates, KPC-positive *K. pneumoniae* ATCC BAA 1705 and NDM-positive *K. pneumoniae* NCTC 13443, were made in saline with a starting inoculum equivalent to a 0.5 McFarland standard. Quantitative culture was performed on the final dilution. Whole organisms in each dilution series (90,000 to 0.9 CFU/μl for KPC-positive *K. pneumoniae* ATCC BAA 1705, and 190,000 to 1.9 CFU/μl for NDM-positive *K. pneumoniae* NCTC 13443) were then heat killed and spiked into perianal swabs (BBL Culture-Swab with Stuart's medium; Becton Dickinson, Franklin Lakes, NJ), and stool in 50% Stool Recovery and Transport (STAR) medium (Roche Applied Science, Indianapolis, IN). The stool specimens were originally collected in Cary-Blair transport medium (Para-Pak C&S; Meridian Bioscience, Cincinnati, OH); a cotton swab was used to inoculate stool into 1-ml aliquots of STAR medium. The spiked stool specimens in STAR medium were replated in triplicate on the MagNA Pure LC 1.0 instrument using the MagNA Pure total nucleic acid protocol/kit (Roche Applied Science). The spiked specimens were tested by the duplex bla_{KPC} and bla_{NDM} PCR assay, a real-time fluorescent resonance energy transfer (FRET) hybridization probe-based real-time PCR assay targeting a 160-bp region of the bla_{KPC-2} gene and a 156-bp region of the bla_{NDM-1} gene, performed on the LightCycler 2.0 instrument (Roche Molecular Diagnostics, Indianapolis, IN) (25).

Accuracy studies (clinical surveillance swabs). (i) Sensitivity panel. We tested a sensitivity panel consisting of 47 previously characterized perirectal double-swab specimens (Becton Dickinson) that were determined at the Rush University Medical Center, Chicago, to be culture positive for bla_{KPC} PCR-positive *Enterobacteriaceae* using the direct ertapenem disk screening method described by Lolans et al. (9) and a bla_{KPC} PCR assay described by Cole et al. (26), which was modified to include a 16S rRNA gene target as an internal control (18). These perirectal surveillance swabs were collected between September and November 2012 as

part of a longitudinal study on CPE colonization in patients in Chicago long-term acute care facilities (27). The perirectal swabs were inserted approximately 1 cm past the anal verge and obtained by study personnel. One swab from each double-swab specimen was plated during this initial screen and then aseptically replaced in the original transport tube containing a sponge reservoir with Stuart's transport medium. The swabs were stored at 4°C and tested for this study within 10 to 41 days (median, 20 days) of collection.

(ii) Specificity panel. The specificity panel consisted of 80 self- or provider-collected perianal double swabs obtained from consecutively enrolled international patients hospitalized at the Mayo Clinic, Rochester, MN, from February to April 2013 as part of a study assessing CPE carriage in at-risk patients (institutional review board [IRB] no. 12-008283). These swabs were stored at 4°C and tested within 7 days of collection.

Testing of clinical surveillance swabs. The general protocol for the testing of clinical surveillance swabs is outlined in Fig. 1 and described in detail below.

Direct PCR method. The first swab (swab 1) from each specimen was placed in a LightCycler Advanced lysis tube (Roche Molecular Diagnostics) and subjected to heat lysis on a Thermomixer R (Eppendorf AG, Germany) for 6 min at 99°C and 1,400 rpm, followed by centrifugation at 20,800 × *g* for 20 s. Five microliters of supernatant was added to 15 μl of PCR mastermix in a 20-μl LightCycler reaction cuvette and tested with the duplex bla_{KPC}-bla_{NDM} assay (25).

HardyCHROM ESBL agar. The second swab (swab 2) was first plated by rotating it 360° across the first quadrant of a HardyCHROM ESBL plate. The plate was streaked for isolation to four quadrants using a sterile loop. The plates were incubated at 35°C in ambient air for 18 to 24 h and observed for growth. If no growth was observed at 24 h, the plates were reincubated for another 24 h, according to the manufacturer's instructions. All morphologically distinct colonies were identified using Bruker Biotyper matrix-assisted laser desorption–time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Billerica, MA) and tested for antimicrobial susceptibility using agar dilution. The bla_{KPC}-bla_{NDM} assay and the MHT were performed on isolates that were carbapenem resistant according to CLSI criteria (28).

Broth-enriched PCR (BE-PCR). After plating on HC-A, swab 2 was placed into tryptic soy broth (TSB) (BD BBL; Becton Dickinson) with a 10-μg meropenem disk (BD BBL Sensi-Disc; Becton Dickinson), vortexed for 5 s, and incubated for 18 to 24 h at 35°C in ambient air with loosened caps. A 6-μl aliquot of the TSB, after incubation, was heat lysed in the lysis tube, as described above, and tested with the duplex PCR assay.

CDC method for CPE screening. The CDC-A was performed using the protocol recommended by the CDC (10), with a slight modification in that eosin methylene blue (EMB) agar (Becton Dickinson) was used instead of MacConkey agar. Briefly, a 100-μl aliquot of the TSB incubated overnight with swab 2 was plated on EMB and streaked for isolation. The plate was incubated for 18 to 24 h at 35°C in ambient air. All morphologically distinct colonies were tested with the duplex PCR assay, identified using MALDI-TOF MS, and subjected to agar dilution antibiotic susceptibility testing. In addition, the MHT was performed on isolates from the sensitivity panel.

Categorization of isolates for analysis. For the purposes of this analysis, the isolates were considered by species and susceptibility according to breakpoints defined by the CLSI (28). Resistance was classified into three categories: (i) carbapenem-resistant GNB (CR-GNB), defined as *Enterobacteriaceae* that were nonsusceptible to either ertapenem (MIC > 0.5 μg/ml) or meropenem (MIC > 1 μg/ml) and nonfermenting GNB that were nonsusceptible to meropenem (MIC > 2 μg/ml for *Pseudomonas aeruginosa* and MIC > 4 μg/ml for other nonfermenting GNB); (ii) third- or fourth-generation cephalosporin (oxyimino-cephalosporin)-resistant GNB (CephR-GNB), defined as *Enterobacteriaceae* that were nonsusceptible to ceftriaxone (MIC > 1 μg/ml), ceftazidime (MIC > 4 μg/ml), or cefepime (MIC > 8 μg/ml) and nonfermenting GNB that were nonsus-
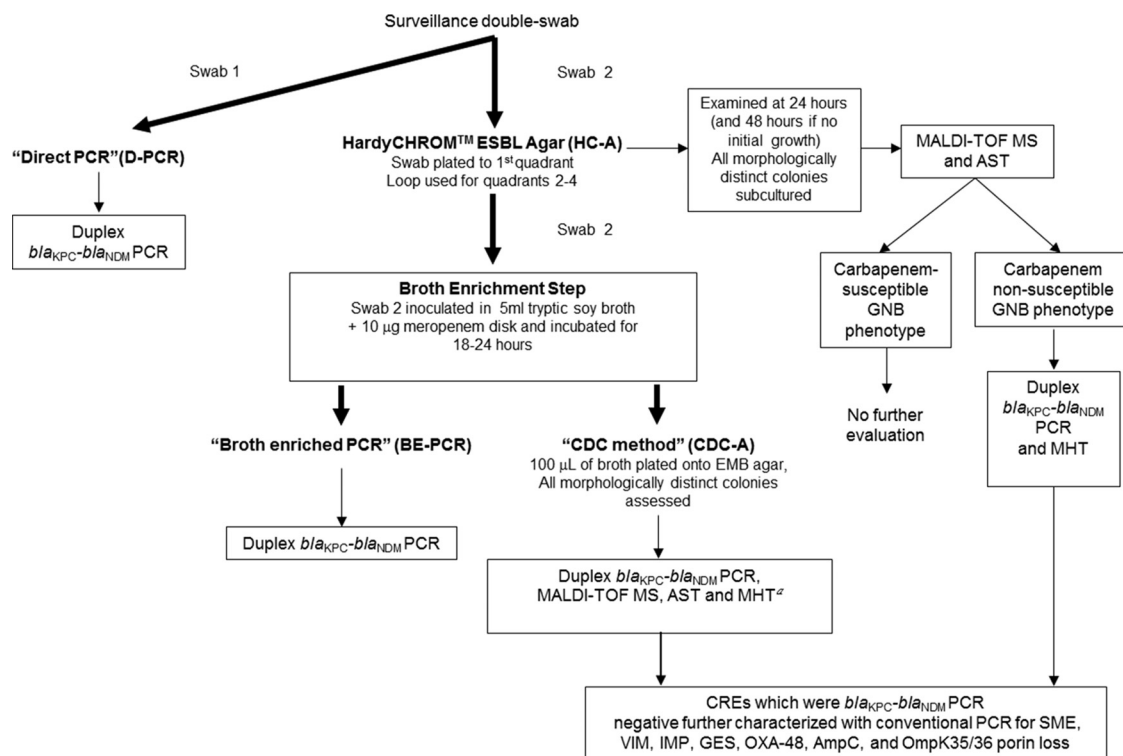
FIG 1 Study schema for clinical surveillance swabs. MALDI-TOF MS, matrix-assisted laser desorption–time of flight mass spectrometry; AST, antimicrobial susceptibility testing (by agar dilution); CRE, carbapenem-resistant *Enterobacteriaceae* (per CLSI 2012 interpretative criteria); EMB agar, eosin methylene blue agar; MHT, modified Hodge test; GNB, Gram-negative bacilli. MHT was performed on isolates from the sensitivity panel ([a]).

ceptible to for ceftriaxone, ceftazidime, and cefepime (all MIC > 8 μg/ml); only ceftazidime and cefepime were considered for *P. aeruginosa* isolates; (iii) susceptible GNB (S-GNB), defined as any GNB that was fully susceptible to the tested carbapenems and oxyimino-cephalosporins.

Further molecular characterization of carbapenem-resistant *Enterobacteriaceae* isolates negative for bla_{KPC} and bla_{NDM}. If negative for bla_{KPC} and bla_{NDM}, carbapenem-resistant *Enterobacteriaceae* isolates from HC-A and CDC-A were further evaluated for SME (29), VIM (30), IMP (31), GES (32), OXA-48 (33), AmpC (34), and OmpK35/36 porin loss (35) using conventional PCR. Molecular confirmation of the presence of carbapenemase genes, either by the real-time bla_{KPC}-bla_{NDM} assay or conventional PCR, was considered to be the gold standard for assigning a CPE status.

Accuracy studies (spiked specimens). In order to supplement the clinical sample data, blinded randomized spiking studies were performed using perianal swabs and Cary-Blair transported stool (predetermined to be negative for bla_{KPC} and bla_{NDM}). Briefly, samples were spiked with heat-killed organisms near the limit of detection of the bla_{KPC}-bla_{NDM} assay. This corresponded to 395 CFU/μl of *K. pneumoniae* NCTC 13443 (bla_{NDM}) and/or 550 CFU/μl *K. pneumoniae* ATCC BAA-1705 (bla_{KPC}). The spiked samples were then assayed with the duplex bla_{KPC}-bla_{NDM} assay with swabs processed with the D-PCR method, and with stool specimens that had undergone extraction prior to PCR.

Statistical analysis. Data analysis was performed using SAS version 9.3 (SAS Institute Inc., Cary, NC). McNemar's test was used for comparisons of paired variables. A P value of <0.05 was considered significant.

RESULTS

Limit of detection. The limit of detection of the PCR assay was 9 CFU/μl and 90 CFU/μl for bla_{KPC} in perianal swabs and stool samples, respectively, and 1.9 CFU/μl for bla_{NDM} in both perianal swabs and stool.

Accuracy studies (clinical surveillance swabs). (i) Molecular methods (D-PCR and BE-PCR). For the sensitivity panel, 47 swabs, each collected from an individual patient and previously determined to be culture positive for bla_{KPC} *Enterobacteriaceae*, were tested. One swab was negative by all four methods (both molecular and culture-based methods; the culture yielded no growth at all), implying the loss of organisms with possible initial low inoculum; this specimen was excluded from the analysis. Of the remaining 46 swabs assessed, D-PCR and BE-PCR were positive for 41/46 (89.1%) and 43/46 swabs (93.5%) without prior extraction. The swabs that were initially negative by D-PCR

TABLE 1 Clinical sensitivity of the evaluated methods for detecting bla_{KPC} in rectal surveillance swabs

Swab testing method	No. of positive swabs/total no. of swabs	Sensitivity (% [95% confidence interval])[c]
Direct PCR[a]	46/46	100 (92.3–100)
Broth-enriched PCR[a]	46/46	100 (92.3–100)
CDC method[b]	36/46	78.3 (64.4–78.7)
HardyCHROM ESBL agar[b]	35/46	76.1 (62.11–86.1)

[a] Five swabs with the D-PCR and 3 swabs with the BE-PCR with visible soiling were negative on initial testing, but were positive on retesting after nucleic acid extraction on the MagNA Pure Compact system.
[b] For the CDC method, bla_{KPC} and bla_{NDM} PCR was performed on all morphologically distinct colonies. For the HardyCHROM ESBL agar method, PCR was performed on carbapenem-resistant isolates. A specimen was considered positive if any isolate from that specimen was bla_{KPC} positive by PCR.
[c] McNemar's test D/BE-PCR versus HC-A, $P = 0.0009$; D/BE-PCR versus CDC-A, $P = 0.0016$; CDC-A versus HC-A, $P = 0.76$.

TABLE 2 Breakdown of cultured Gram-negative bacilli from bla_{KPC}-positive rectal surveillance swabs by culture method, identification, susceptibility phenotype, and bla_{KPC} status

Culture method(s) detection[a]	Organism characteristics		No. with susceptibility phenotype[b]:				bla_{KPC} PCR-positive (% of CR-GNB)[c]
	Nonfermenter or Enterobacteriaceae	Genus/species	CR-GNB	CephR-GNB	S-GNB	Total (all susceptibility phenotypes)	
CDC-A and HC-A	Nonfermenter	Acinetobacter spp.	2	1	0	3	0 (0)
		Pseudomonas aeruginosa	10	2	2	14	0 (0)
	Enterobacteriaceae	Escherichia coli	1	4	0	5	1 (100)
		Klebsiella pneumoniae	28	2	0	30	28 (100)
		Morganella morganii	0	1	0	1	
		Proteus mirabilis	0	1	0	1	
		Providencia stuartii	0	2	0	2	
CDC-A alone	Nonfermenters	Acinetobacter spp.	3	0	0	3	0 (0)
		Pseudomonas aeruginosa	7	1	6	14	0 (0)
		Alcaligenes faecalis	0	0	1	1	
	Enterobacteriaceae	Citrobacter spp.	1	0	0	1	1 (100)
		E. coli	1	0	5	6	0 (0)
		K. pneumoniae	4	1	1	6	4 (100)
		M. morganii	0	0	1	1	
		P. mirabilis	0	2	5	7	
		P. stuartii	1	1	3	5	1 (100)
HC-A alone	Nonfermenters	Acinetobacter spp.	2	0	0	2	0 (0)
		P. aeruginosa	2	0	0	2	0 (0)
	Enterobacteriaceae	Citrobacter spp.	0	3	0	3	
		Enterobacter aerogenes	1	0	0	1	1 (100)
		Enterobacter cloacae complex	2	1	0	3	0 (0)
		E. coli	3	8	0	11	0 (0)
		K. pneumoniae	6	8	0	14	6 (100)
		M. morganii	0	4	1	5	
		P. mirabilis	0	4	0	4	
		P. stuartii	0	2	0	2	
		Serratia marcescens	0	1	0	1	
Total	Nonfermenters	Acinetobacter spp.	7	1	0	8	0 (0)
		A. faecalis	0	0	1	1	
		P. aeruginosa	19	3	8	30	0 (0)
	Enterobacteriaceae	Citrobacter spp.	1	3	0	4	1 (100)
		E. aerogenes	1	0	0	1	1 (100)
		E. cloacae complex	2	1	0	3	0 (0)
		E. coli	5	12	5	22	1 (20)
		K. pneumoniae	38	11	1	50	38 (100)
		M. morganii	0	5	2	7	
		P. mirabilis	0	7	5	12	
		P. stuartii	1	5	3	9	1 (100)
		S. marcescens	0	1	0	1	
All genera/species			74	49	25	148	42 (56.8)

[a] CDC-A: CDC-recommended method; HC-A: HardyCHROM Agar ESBL.
[b] CR-GNB, carbapenem-resistant GNB; CephR-GNB, 3rd- or 4th-generation cephalosporin-resistant GNB (oxyimino-cephalosporin, i.e., ceftriaxone, ceftazidime, or cefepime). For P. aeruginosa isolates, only ceftazidime and cefepime were considered. S-GNB, susceptible GNB (susceptible to carbapenems or oxyimino-cephalosporins). Isolates testing "intermediate" in susceptibility were classified together with resistant isolates. See "Categorization of isolates for analysis" in the text for detailed definitions.
[c] bla_{KPC} and bla_{NDM} PCR was performed for all isolates cultured by CDC-A and for carbapenem-nonsusceptible isolates cultured by HC-A for the sensitivity panel. All isolates from the clinical surveillance swabs that were bla_{KPC} positive in this study were CR-GNB.

($n = 5$) and BE-PCR ($n = 3$) were visibly soiled with stool, and all were positive for bla_{KPC} upon repeat testing after the lysates were extracted. Thus, in an approach in which swabs visibly soiled with stool underwent extraction prior to PCR, both the D-PCR and BE-PCR yielded 100% clinical sensitivity (Table 1). All 80 swabs in the specificity panel tested negative by D-PCR and BE-PCR.

(ii) Culture-based methods (HC-A and CDC-A). Overall, when considered by species and susceptibility categories, 148 distinct isolates were cultured from the 46 swabs previously determined to be culture positive for bla_{KPC} Enterobacteriaceae, 56 by both HC-A and CDC-A, 44 by CDC-A alone, and 48 by HC-A alone. Of the 148 isolates, 109 were Enterobacteriaceae and 39 were

TABLE 3 bla_{KPC} and modified Hodge test status by susceptibility phenotype and culture method in Gram-negative bacilli cultured from bla_{KPC}-positive rectal surveillance swabs

Testing method and results by GNB group	Data by modified Hodge test results:		
	Positive	Negative	Noninterpretable
CDC method			
Carbapenem-resistant Gram-negative bacilli ($n = 58$)			
bla_{KPC} PCR positive	35	0	0
bla_{KPC} PCR negative	7	14	2
3rd-/4th-generation (oxyimino-cephalosporin)-resistant Gram-negative bacilli ($n = 18$)			
bla_{KPC} PCR positive	0	0	0
bla_{KPC} PCR negative	2	15	1
Susceptible Gram-negative bacilli ($n = 24$)			
bla_{KPC} PCR positive	0	0	0
bla_{KPC} PCR negative	2	17	5
HardyCHROM ESBL			
Carbapenem-resistant Gram-negative bacilli ($n = 57$)			
bla_{KPC} PCR positive	36	0	0
bla_{KPC} PCR negative	8	10	3

nonfermenting GNB; 74 were CR-GNB, 49 were CephR-GNB, and 25 were S-GNB (Table 2).

CDC-A yielded 100 distinct isolates, including 58 CR-GNB and 42 carbapenem-susceptible GNB (18 CephR-GNB and 24 S-GNB). Of the 100 isolates, the MHT was positive for 46 and the bla_{KPC} PCR positive for 35 of 46 (76.1%) (Table 3). HC-A yielded 104 distinct isolates, including 57 CR-GNB and 47 carbapenem-susceptible GNB (44 CephR-GNB and 3 S-GNB). Of the 57 CR-GNBs, 44 (77.2%) were MHT positive, of which 36 (81.8%) were bla_{KPC} PCR positive. The bla_{KPC}-positive isolates had MIC$_{50}$s of >4 µg/ml to ertapenem and 8 µg/ml to meropenem by both plate methods. The other carbapenem-resistant *Enterobacteriaceae* in the sensitivity panel that were negative for bla_{KPC} and bla_{NDM} were also negative for the other less commonly encountered carbapenemases assessed (Table 4).

Of 80 swabs in the specificity panel, one carbapenem-resistant *Enterobacteriaceae* isolate was cultured from one specimen (by both CDC-A and HC-A). This was an *Escherichia coli* isolate with a meropenem MIC of 2 µg/ml and an ertapenem MIC of >4 µg/ml. The isolate was positive for AmpC and negative by MHT, bla_{KPC}/bla_{NDM} PCR, and the other less commonly encountered carbapenemases tested. This isolate was designated a non-CPE (Table 4). Thus, in the specificity panel, no CPE were detected by any evaluated method (100% agreement).

Overall, the sensitivities of the culture-based methods studied were inferior to that of PCR: the sensitivity of the CDC-A was 78.3%, versus 100% for PCR ($P = 0.0016$) and the sensitivity of HC-A was 76.1% versus 100% for PCR ($P = 0.0009$).

Accuracy studies (spiked specimens). The sensitivities of bla_{KPC} PCR in spiked surveillance swabs and stool were 93.3% and 100%, respectively. The sensitivity of bla_{NDM} PCR in both spiked surveillance swabs and stool was 100% (data not shown).

DISCUSSION

In this study, we demonstrated that our assay (D-PCR) accurately detected bla_{KPC} and bla_{NDM} directly from perirectal and perianal swabs. We believe that NAAT testing performed directly from surveillance specimens for the detection of CPE represents the most sensitive and straightforward methodology. The majority of the clinical surveillance swabs in our study were not visibly soiled with stool and were able to be processed with a simple lysis procedure, without upfront extraction, which saves money and shortens the turnaround time. Additionally, we found that compared to D-PCR, a broth enrichment step (BE-PCR) did not improve sensitivity or completely eliminate inhibition in swabs that were visibly soiled with stool. Our approach using D-PCR without extraction has a turnaround time of 90 min from swab collection to the results and can be performed for the majority of swab specimens. Nucleic acid extraction should be performed on swabs that are soiled with stool or if testing is performed directly on stool. D-PCR is anticipated to allow for accurate and rapid test results and will be especially helpful in outbreak settings where screening volumes are increased and the timely implementation of infection control measures is paramount. When isolates are required for

TABLE 4 Characteristics of carbapenem-resistant *Enterobacteriaceae* isolated from clinical surveillance swabs that were negative for bla_{KPC} and bla_{NDM}

Isolate no.	Panel/method isolated from[a]:	Species	Ertapenem/meropenem MIC (µg/ml)	Results by:		Other resistance mechanisms
				Modified Hodge test	bla_{KPC}/bla_{NDM} PCR	
1	Sensitivity panel, CDC-A	*Escherichia coli*	$>4/>8$	Negative	Negative	Unknown mechanism, ESBL phenotype
2	Sensitivity panel, HC-A	*E. coli*	$>4/\leq1$	Negative	Negative	Unknown mechanism, ESBL phenotype
3	Sensitivity panel, HC-A	*E. coli*	$1/\leq1$	Inhibited (noninterpretable)	Negative	AmpC
4	Sensitivity panel, HC-A	*Enterobacter cloacae* complex	$1/\leq1$	Positive (ertapenem disk positive, meropenem disk weak positive)	Negative	Unknown mechanism, ESBL phenotype
5	Sensitivity panel, HC-A	*E. coli*	$>4/8$	Positive (ertapenem and meropenem disks)	Negative	AmpC
6	Sensitivity panel, HC-A	*E. cloacae* complex	$1/\leq1$	Positive (ertapenem and meropenem disks)	Negative	AmpC
7	Specificity panel, HC-A	*E. coli*	$>4/2$	Negative	Negative	AmpC

[a] CDC-A, CDC method; HC-A, HardyCHROM ESBL.

further characterization, cultures can be performed from a second swab (if double swabs are used) or from the sponge from the swab-holding tube.

The D-PCR approach was more sensitive than the two culture methods we assessed, the widely used CDC-recommended method (CDC-A) and HardyCHROM ESBL agar (HC-A) method. The CDC-A was labor-intensive and had a long turnaround time. Despite the selective broth incubation step with meropenem, a substantial amount of breakthrough growth of carbapenem-susceptible isolates was noted with the CDC-A (42% of isolates cultured by CDC-A). We chose to use an extended-spectrum β-lactamase (ESBL) selective chromogenic medium (HC-A) for our study, as this detects both ESBLs and CPE and is theoretically more sensitive than carbapenem-resistant *Enterobacteriaceae* selective media, especially for CPE with low carbapenem MICs. However, the performance of the D-PCR/BE-PCR was superior to that of the HC-A. The HC-A is easy to read because colony color allows for the presumptive identification of an organism as *E. coli* (pink) or *Klebsiella/Enterobacter* species (blue), but like in the CDC-A, isolated colonies require further testing (e.g., MHT, antimicrobial susceptibility testing) to determine CPE status, and molecular testing is required for confirmation and characterization.

There were several limitations to our study. First, the prevalence of CPE is low at Mayo Clinic, and so part of this study was performed using spiked specimens. Accuracy studies were performed using swabs previously shown to be culture positive for bla_{KPC}-positive *Enterobacteriaceae* that were collected in a region where bla_{KPC} is endemic; there was a median time interval of 20 days from when these swabs were collected to when this study was done. We believe that there was minimal loss of viability of bacteria on the swabs and a consequent bias against the culture-based methods studied, as the swabs were stored at 4°C, and all except one (which was negative by all four methods and thus was excluded) yielded viable bacteria (although not always CPE). Additionally, all bla_{KPC}-positive swabs that were negative by the HC-A were detected by the CDC-A (and vice versa), except for five swabs. A second limitation is that bla_{NDM}, while reported, is not endemic in the United States, and as such we were unable to assess the clinical accuracy of detection of this carbapenemase in our clinical surveillance swab panel. However, the accuracy as assessed by spiking studies performed near the limit of detection showed excellent (100%) sensitivity for bla_{NDM}.

Emerging and novel carbapenemases will likely make the detection of CPE a challenge and a "moving goal post," so it is important to keep abreast of and be able to detect the most prevalent CPE mechanisms in one's area of practice. For example, a recent hospital outbreak of bla_{NDM} was reported in Denver (36) where affected patients had not traveled internationally and it was not clear how bla_{NDM} was introduced. It may be increasingly difficult to differentiate bla_{NDM} and bla_{KPC} carriers by their type of exposure, thus justifying the need for rapid and accurate tests that can differentiate between the carbapenemases. While culture-based methods can be considered truly multiplexed in that various CPE may potentially be detected, we did not detect other more rarely occurring carbapenemases in our study (Table 4). Institutions should screen for the carbapenemases that are most commonly encountered in their areas of practice. In the United States, this would include routine assessment for bla_{NDM} and bla_{KPC} at a minimum, as is recommended by the CDC (4). Isolates suspected of

being CPE and that are negative for bla_{NDM} and bla_{KPC} should be referred to public health laboratories for further characterization.

In summary, PCR performed directly on perirectal and perianal surveillance swab specimens after a simple lysis procedure (when swabs are not visibly soiled with stool) provides timely and accurate results. This approach can also be applied to stool and swabs soiled with stool after a nucleic acid extraction step. Where laboratory resources allow, we believe that NAAT testing performed directly on surveillance specimens represents the most sensitive and straightforward screening approach for the detection of CPE.

ACKNOWLEDGMENTS

REFERENCES

1. **Kochar S, Sheard T, Sharma R, Hui A, Tolentino E, Allen G, Landman D, Bratu S, Augenbraun M, Quale J.** 2009. Success of an infection control program to reduce the spread of carbapenem-resistant *Klebsiella pneumoniae*. Infect. Control Hosp. Epidemiol. **30:**447–452.
2. **Munoz-Price LS, Hayden MK, Lolans K, Won S, Calvert K, Lin M, Stemer A, Weinstein RA.** 2010. Successful control of an outbreak of *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* at a long-term acute care hospital. Infect. Control Hosp. Epidemiol. **31:**341–347.
3. **Chitnis AS, Caruthers PS, Rao AK, Lamb J, Lurvey R, Beau De Rochars V, Kitchel B, Cancio M, Török TJ, Guh AY, Gould CV, Wise ME.** 2012. Outbreak of carbapenem-resistant *Enterobacteriaceae* at a long-term acute care hospital: sustained reductions in transmission through active surveillance and targeted interventions. Infect. Control Hosp. Epidemiol. **33:**984–992.
4. **Centers for Disease Control and Prevention Health Alert Network.** 2013. CDC health advisory: new carbapenem-resistant enterobacteriaceae warrant additional action by healthcare providers. CDC Health Alert Network, Atlanta, GA. http://www.bt.cdc.gov/HAN/han00341asp.
5. **Panagea T, Galani I, Souli M, Adamou P, Antoniadou A, Giamarellou H.** 2011. Evaluation of CHROMagar KPC for the detection of carbapenemase-producing *Enterobacteriaceae* in rectal surveillance cultures. Int. J. Antimicrob. Agents **37:**124–128.
6. **Adler A, Navon-Venezia S, Moran-Gilad J, Marcos E, Schwartz D, Carmeli Y.** 2011. Laboratory and clinical evaluation of screening agar plates for detection of carbapenem-resistant *Enterobacteriaceae* from surveillance rectal swabs. J. Clin. Microbiol. **49:**2239–2242.
7. **Girlich D, Poirel L, Nordmann P.** 2013. Comparison of the SUPERCARBA, CHROMagar KPC, and Brilliance CRE screening media for detection of *Enterobacteriaceae* with reduced susceptibility to carbapenems. Diagn. Microbiol. Infect. Dis. **75:**214–217.
8. **Samra Z, Bahar J, Madar-Shapiro L, Aziz N, Israel S, Bishara J.** 2008. Evaluation of CHROMagar KPC for rapid detection of carbapenem-resistant *Enterobacteriaceae*. J. Clin. Microbiol. **46:**3110–3111.
9. **Lolans K, Calvert K, Won S, Clark J, Hayden MK.** 2010. Direct ertapenem disk screening method for identification of KPC-producing *Klebsiella pneumoniae* and *Escherichia coli* in surveillance swab specimens. J. Clin. Microbiol. **48:**836–841.
10. **Centers for Disease Control and Prevention.** 2008. Laboratory protocol for detection of carbapenem-resistant or carbapenemase-producing, *Klebsiella* spp. and *E. coli* from rectal swabs. U.S. Department of Health and Human Services, CDC, Atlanta, GA.
11. **Girlich D, Poirel L, Nordmann P.** 2012. Value of the modified Hodge test for detection of emerging carbapenemases in *Enterobacteriaceae*. J. Clin. Microbiol. **50:**477–479.
12. **Snitkin ES, Zelazny AM, Thomas PJ, Stock F, NISC Comparative Sequencing Program Group, Henderson DK, Palmore TN, Segre JA.** 2012. Tracking a hospital outbreak of carbapenem-resistant *Klebsiella pneumoniae* with whole-genome sequencing. Sci. Transl. Med. **4:**148ra116. doi:10.1126/scitranslmed.3004129.
13. **Lau A, Stock F, Palmore T, Zelazny A.** 2012. Laboratory response to a

KPC outbreak at the NIH Clinical Center. Abstr. 112th Gen. Meet. Am. Soc. Microbiol., 16–19 June 2012, San Francisco, CA.

14. **Hindiyeh M, Smollen G, Grossman Z, Ram D, Davidson Y, Mileguir F, Vax M, Ben David D, Tal I, Rahav G, Shamiss A, Mendelson E, Keller N.** 2008. Rapid detection of *bla*_{KPC} carbapenemase genes by real-time PCR. J. Clin. Microbiol. **46:**2879–2883.

15. **Schechner V, Straus-Robinson K, Schwartz D, Pfeffer I, Tarabeia J, Moskovich R, Chmelnitsky I, Schwaber MJ, Carmeli Y, Navon-Venezia S.** 2009. Evaluation of PCR-based testing for surveillance of KPC-producing carbapenem-resistant members of the *Enterobacteriaceae* family. J. Clin. Microbiol. **47:**3261–3265.

16. **Naas T, Cotellon G, Ergani A, Nordmann P.** 2013. Real-time PCR for detection of *bla*_{OXA-48} genes from stools. J. Antimicrob. Chemother. **68:** 101–104.

17. **Naas T, Ergani A, Carrer A, Nordmann P.** 2011. Real-time PCR for detection of NDM-1 carbapenemase genes from spiked stool samples. Antimicrob. Agents Chemother. **55:**4038–4043.

18. **Mangold KA, Santiano K, Broekman R, Krafft CA, Voss B, Wang V, Hacek DM, Usacheva EA, Thomson RB, Jr, Kaul KL, Peterson LR.** 2011. Real-time detection of *bla*_{KPC} in clinical samples and surveillance specimens. J. Clin. Microbiol. **49:**3338–3339.

19. **Singh K, Mangold KA, Wyant K, Schora DM, Voss B, Kaul KL, Hayden MK, Chundi V, Peterson LR.** 2012. Rectal screening for *Klebsiella pneumoniae* carbapenemases: comparison of real-time PCR and culture using two selective screening agar plates. J. Clin. Microbiol. **50:**2596–2600.

20. **Richter SN, Frasson I, Biasolo MA, Bartolini A, Cavallaro A, Palù G.** 2012. Ultra-rapid detection of *bla*_{KPC1/2-12} from perirectal and nasal swabs by use of real-time PCR. J. Clin. Microbiol. **50:**1718–1720.

21. **McEwan AS, Derome A, Meunier D, Burns PJ, Woodford N, Dodgson AR.** 2013. Evaluation of the NucliSENS EasyQ KPC assay for detection of *Klebsiella pneumoniae* carbapenemase-producing *Enterobacteriaceae*. J. Clin. Microbiol. **51:**1948–1950.

22. **Avlami A, Bekris S, Ganteris G, Kraniotaki E, Malamou-Lada E, Orfanidou M, Paniara O, Pantazatou A, Papagiannitsis CC, Platsouka E, Stefanou I, Tzelepi E, Vagiakou H, Miriagou V.** 2010. Detection of metallo-β-lactamase genes in clinical specimens by a commercial multiplex PCR system. J. Microbiol. Methods **83:**185–187.

23. **Kaase M, Szabados F, Wassill L, Gatermann SG.** 2012. Detection of carbapenemases in *Enterobacteriaceae* by a commercial multiplex PCR. J. Clin. Microbiol. **50:**3115–3118.

24. **Nijhuis RHT, Savelkoul PHM, van Zwet AA.** 2013. A new real-time PCR for rapid detection of VIM, OXA-48, NDM and KPC carbapenemases in Gram negative bacteria directly from rectal swabs. Abstract P-1846. 23rd European Congress of Clinical Microbiology and Infectious Diseases, 27–30 April 2013, Berlin, Germany.

25. **Cunningham SA, Noorie T, Meunier D, Woodford N, Patel R.** 2013. Rapid and simultaneous detection of genes encoding *Klebsiella pneu-moniae* carbapenemase (*bla*_{KPC}) and New Delhi metallo-β-lactamase (*bla*_{NDM}) in Gram-negative bacilli. J. Clin. Microbiol. **51:**1269–1271.

26. **Cole JM, Schuetz AN, Hill CE, Nolte FS.** 2009. Development and evaluation of a real-time PCR assay for detection of *Klebsiella pneumoniae* carbapenemase genes. J. Clin. Microbiol. **47:**322–326.

27. **Thurlow CJ, Prabaker K, Lin MY, Lolans K, Weinstein RA, Hayden MK, Centers for Disease Control and Prevention Epicenters Program.** 2013. Anatomic sites of patient colonization and environmental contamination with *Klebsiella pneumoniae* carbapenemase-producing *Enterobacteriaceae* at long-term acute care hospitals. Infect. Control Hosp. Epidemiol. **34:**56–61.

28. **Clinical and Laboratory Standards Institute.** 2013. Performance standards for antimicrobial susceptibility testing; 23rd informational supplement. CLSI M100-S23. Clinical and Laboratory Standards Institute, Wayne, PA.

29. **Hong SS, Kim K, Huh JY, Jung B, Kang MS, Hong SG.** 2012. Multiplex PCR for rapid detection of genes encoding class A carbapenemases. Ann. Lab. Med. **32:**359–361.

30. **Poirel L, Naas T, Nicolas D, Collet L, Bellais S, Cavallo JD, Nordmann P.** 2000. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-beta-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. Antimicrob. Agents Chemother. **44:**891–897.

31. **Senda K, Arakawa Y, Ichiyama S, Nakashima K, Ito H, Ohsuka S, Shimokata K, Kato N, Ohta M.** 1998. PCR detection of metallo-β-lactamase gene (*bla*_{IMP}) in Gram-negative rods resistant to broad-spectrum β-lactams. J. Clin. Microbiol. **34:**2909–2913.

32. **Poirel L, Le Thomas I, Naas T, Karim A, Nordmann P.** 2000. Biochemical sequence analyses of GES-1, a novel class A extended-spectrum beta-lactamase, and the class 1 integron In52 from *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **44:**622–632.

33. **Poirel L, Héritier C, Tolün V, Nordmann P.** 2004. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **48:**15–22.

34. **Pérez-Pérez FJ, Hanson ND.** 2002. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J. Clin. Microbiol. **40:**2153–2162.

35. **Kaczmarek FM, Dib-Hajj F, Shang W, Gootz TD.** 2006. High-level carbapenem resistance in a *Klebsiella pneumoniae* clinical isolate is due to the combination of *bla*_{ACT-1} beta-lactamase production, porin OmpK35/36 insertional inactivation, and down-regulation of the phosphate transport porin PhoE. Antimicrob. Agents Chemother. **50:**3396–3406.

36. **Centers for Disease Control and Prevention.** 2013. Notes from the field: hospital outbreak of carbapenem-resistant *Klebsiella pneumoniae* producing New Delhi metallo-beta-lactamase—Denver, Colorado, 2012. MMWR Morb. Mortal. Wkly. Rep. **62:**108.