Multicenter Evaluation of the Xpert Carba-R Assay for Detection of Carbapenemase Genes in Gram-Negative Isolates

Maria M. Traczewski,[a] Edoardo Carretto,[b] Rafael Canton,[c] Nicholas M. Moore,[d] for the Carba-R Study Team

[a]Clinical Microbiology Institute, Wilsonville, Oregon, USA
[b]Clinical Microbiology Laboratory, IRCCS Arcispedale Santa Maria Nuova, Reggio Emilia, Italy
[c]Servicio de Microbiología, Hospital Ramón y Cajal and Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain
[d]Rush University Medical Center, Chicago, Illinois, USA

ABSTRACT This multicenter study evaluated the performance of the Cepheid Xpert Carba-R assay, a qualitative PCR test designed for the rapid detection of bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, and bla_{OXA-48} carbapenem resistance genes from bacterial isolates grown on blood agar or MacConkey agar. The results were compared to those obtained from bidirectional DNA sequence analysis of nucleic acid extracted from pure colonies. Isolates of *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* that tested as either intermediate or resistant to a carbapenem antibiotic were analyzed. A total of 467 isolates were evaluated, including prospectively collected clinical isolates, frozen isolates, and a group of contrived broth specimens sent by a central reference laboratory. The assay was run on the Gene-Xpert platform and took 48 min, with less than 1 min of hands-on time. Compared to the results of the reference methods, the overall sensitivity of the assay was 100% (95% confidence interval [CI], 99.0 to 100%) for isolates grown on both blood and MacConkey agars. Overall specificity was 98.1% (95% CI, 93.1 to 99.8%) and 97.1% (95% CI, 91.7 to 99.4%) for blood and MacConkey agars, respectively. This platform, previously demonstrated to be effective for the detection of carbapenemase genes in rectal swabs, is also adequate for the detection of these genes in bacterial colonies isolated from blood and MacConkey agars.

KEYWORDS carbapenemase detection, molecular methods, Gram-negative bacteria, multidrug resistance, surveillance, Xpert Carba-R assay, bacterial isolates, surveillance studies

The global spread of carbapenem resistance in multiple bacterial genera, including the carbapenemase-producing *Enterobacteriaceae* and carbapenem-resistant isolates of *Pseudomonas aeruginosa* and *Acinetobacter* species, has become a matter of concern worldwide. These bacteria are often resistant to all beta-lactam agents and frequently are coresistant to multiple classes of other antimicrobial agents, leaving few, if any, treatment options (1–3).

Although a variety of species of *Enterobacteriaceae* can harbor carbapenemase genes, the resistance genes are most frequently described in *Klebsiella pneumoniae* and *Escherichia coli* isolates, which can express several different resistance mechanisms. The most common form of resistance in *K. pneumoniae* strains is production of the class A plasmid-encoded carbapenemase bla_{KPC} (4–6). Other resistance genes that have a pivotal role in the spread of carbapenem resistance are those encoding metallo-beta-lactamases (MBLs), such as bla_{VIM}, bla_{NDM}, and bla_{IMP} (7–9). Class D carbapenemases, such as those encoded by the bla_{OXA-48} and $bla_{OXA-48-like}$ genes, which often result in the expression of low-level resistance to carbapenems (10), have been disseminating

among multiple species of *Enterobacteriaceae* and are difficult to detect by phenotypic methods, often because the isolates remain susceptible to extended-spectrum cephalosporins (11). Other enzymes, such as those encoded by bla_{OXA-23} and bla_{OXA-58}, are mainly present in carbapenem-resistant strains of *Acinetobacter baumannii* (12).

The high morbidity and mortality associated with carbapenem-resistant organism (CRO) infections and the rapid spread of the resistant strains causing outbreaks led several countries to adopt screening policies in order to detect asymptomatically colonized patients (13, 14). Screening protocols are mainly based on cultures of rectal or perirectal swab specimens on selective or nonselective media, followed by phenotypic tests to confirm the production and the type of carbapenemases, such as the Carba NP test (15, 16), the carbapenem inactivation method (CIM) (17), or disk diffusion synergy tests (DDST) for detection of MBLs and KPCs (e.g., meropenem disks alone and meropenem disks supplemented with aminophenylboronic acid, dipicolinic acid, or cloxacillin). The addition of a temocillin disk is useful for OXA-48 detection (18). These phenotypic tests are also commonly used as confirmatory tests for clinical specimens with reduced susceptibility to one or more carbapenems as determined by routine antimicrobial susceptibility testing. In general, these methods are inexpensive, are easy to perform, and are likely available in many clinical microbiology laboratories. However, they have several disadvantages: the Carba NP test results are sometimes difficult to evaluate, and invalid results may occur with some isolates that contain carbapenemases, such as OXA-48 or chromosomally encoded OXA enzymes (e.g., *Acinetobacter baumannii*) (19). Furthermore, the CIM method requires at least 6 h to be completed and is unreliable for detecting carbapenemases in *Acinetobacter* species, while DDST also needs prolonged incubation times to complete. Such delays may lead to inappropriate therapy in patients infected by CRO.

Molecular methods have only recently become available for detecting carbapenemase genes directly from clinical specimens. The Cepheid Xpert Carba-R assay (Cepheid, Sunnyvale, CA, USA) is an automated *in vitro* diagnostic test for the qualitative detection of the bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48}, and bla_{IMP} genes, associated with carbapenem nonsusceptibility in Gram-negative bacteria. The assay is performed on the GeneXpert instrument systems and can be performed on rectal swab specimens (a validated U.S. *in vitro* diagnostics [US-IVD] and European Union *in vitro* diagnostics [EU-IVD] method) or on pure cultures of carbapenem-nonsusceptible bacterial isolates (a validated US-IVD method). Results are available in about 48 min (20).

The aim of this study was to evaluate the analytic performance of the Xpert Carba-R assay on bacterial isolates compared to that of reference bidirectional DNA sequence analysis of nucleic acids extracted from pure colonies.

MATERIALS AND METHODS

Study design. This multicenter study was conducted between May 2015 and September 2015 in two health care facilities in the United States (the Rush University Medical Center, Chicago, IL, and the Clinical Microbiology Institute, Wilsonville, OR) and two facilities in Europe (the Hospital Universitario Ramón y Cajal-Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain, and the IRCCS-Arcispedale Santa Maria Nuova, Reggio Emilia, Italy).

All the centers analyzed three different sets of isolates: (i) a set of isolates previously collected from clinical samples (stock isolates), (ii) microorganisms prospectively collected in the different institutions during the study period (fresh isolates), and (iii) a set of additional contrived broth specimens, prepared by seeding well-characterized carbapenem-susceptible and nonsusceptible strains into Mueller-Hinton broth, sent frozen by the reference laboratory (the Clinical Microbiology Institute) to the other centers.

The isolates selected in the different centers (stock and fresh isolates) were *Enterobacteriaceae*, *P. aeruginosa*, and *A. baumannii* isolates that tested either intermediate or resistant to meropenem, ertapenem, and/or imipenem (ertapenem was not considered for *P. aeruginosa* or *A. baumannii*). The stock isolates were clinical isolates stored during the previous 2 years by the different centers at −80°C in microbeads (Pro-Lab Diagnostics, Round Rock, TX, USA), whereas the fresh isolates were analyzed within 7 days from their primary isolation. The evaluation of carbapenem resistance was performed at each center using the automated instruments used as the standard of care, and the results was confirmed through a secondary method based on agar disk diffusion or gradient strips (Etest [bioMérieux, France] and MIC Test Strip [Liofilchem, Italy]).

Xpert Carba-R assay. The selected strains were analyzed after a primary subculture on Trypticase soy agar with 5% sheep blood (BA) and MacConkey agar (MCA) plates. Each plate had a 10-μg meropenem disk added to the first quadrant (to better select resistant strains), and the plates were incubated

overnight at 35°C in ambient air. Well-isolated colonies were diluted in either distilled water or 0.85% saline to the turbidity of a 0.5 McFarland standard, using the direct colony suspension method; no other strain concentrations were tested. The test was performed for colonies grown on both BA and MCA. Ten microliters of the suspension was inoculated into a 5-ml sample reagent vial and vortexed for 30 s. Finally, 1.7 ml of this suspension was transferred into an Xpert Carba-R cartridge using a disposable transfer pipette. The cartridge was loaded onto the GeneXpert system, and the assay was initiated. The instrument operates through the use of GeneXpert Dx software (version 4.3; Cepheid) or Infinity Xpertise software (version 6.1; Cepheid).

If indeterminate results were reported (i.e., the test result was reported as "invalid," "error," or "no result"), a single retest of the sample was performed. If the result of the repeat assay was also indeterminate, it was reported as such. If a valid result was obtained, it was considered for the final analysis.

Quality control for the Xpert Carba-R assay consisted of one bacterial isolate that was positive for all 5 targets of the assay (an *E. coli* strain that contained a plasmid with DNA fragments of all five target gene sequences), one negative isolate (the same *E. coli* strain with the same cloning vector that lacked the inserted gene fragments), and five different positive-control isolates, each of which was positive for a single target of the assay. The negative-control isolate and the positive-control isolate positive for all 5 targets were run on each day that study specimens were tested, along with two of the positive-control isolates harboring a single target. The single-target-positive controls were used on a rotating basis according to a schedule provided in the study protocol. In summary, a total of four controls were run on each day that study specimens were tested. In accordance with good laboratory practices, study specimens were not run until the expected results for the positive and negative controls were obtained.

Data analysis, discrepant result resolution, and statistical analysis. To assess assay performance characteristics, the results of the Xpert Carba-R assay for all the isolates were recorded and compared to those obtained for the reference culture plus the results of DNA sequencing performed in the reference laboratory. A positive result given by the Xpert Carba-R assay indicated the presence of at least one of the target carbapenemase genes in the isolate. For each of the isolates tested, results for each target gene were reported separately and were compared to the individual results for each carbapenemase gene obtained by the reference method.

DNA from the isolates was purified, quantified, and amplified using primers specific to all 5 target genes that amplified regions larger than the assay targets and that included the Xpert Carba-R primer sequences. The presence of amplification products of the expected size was confirmed on an Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). If the bands produced by the bioanalyzer corresponded to any of the 5 target genes detected by the Xpert Carba-R assay, the amplicon for the isolate was sent to an independent laboratory (ACGT, Inc., Wheeling, IL, USA) for reference bidirectional sequencing analysis. If no bands for any of the 5 target genes were produced by the bioanalyzer, the isolate was not sent for sequence analysis and the reference method result was considered negative for the 5 target genes.

Briefly, bidirectional DNA sequencing was performed on isolates from the BA plates, and the resulting consensus sequences for each specimen were compared to the reference sequences. DNA was extracted from a single bacterial colony through the use of the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Germantown, MD, USA), using either a manual extraction protocol or a QIAcube device. Two microliters (approximately 40 ng) of the DNA extracted was then added to a PCR mixture containing primers that were different from those used in the Xpert Carba-R assay and that comprised the Xpert Carba-R assay-targeted sequences. The amplification products were then sent to the ACGT Laboratory for sequencing through the use of an ABI BigDye Terminator (version 3.1) kit (Applied Biosystems, Thermo Fisher Scientific, USA).

Sequences were considered a match if ≥95% sequence similarity was present across the specified target region and the reference method result was considered positive for the target gene(s). The reference method result was considered negative for specimens with <95% similarity across the specified target region. The Xpert Carba-R assay results for isolates from both the BA and MCA cultures were compared to the reference sequencing result for the performance analyses presented.

If the Xpert Carba-R assay results from BA were discordant with the reference sequencing results for a given specimen, the isolate from MCA was sequenced and the result was compared to the Xpert Carba-R assay result. Discrepant sequence analysis was conducted according to the same procedure described above for reference sequencing.

Sample size was calculated on the basis of a power analysis that used a lower 95% confidence interval (CI) of ≥90% for sensitivity, given the hypothesized sensitivity as truth.

RESULTS

A total of 485 isolates (428 clinical stock isolates and 57 fresh isolates) were analyzed. One hundred six isolates were tested at site A, 153 were tested at site B, 98 were tested at site C, and 128 were tested at site D. Two isolates were excluded because reference testing results were not available. Sixteen isolates were further excluded as they were *Pseudomonas* species other than *P. aeruginosa*. The final analysis was performed on 467 isolates: 343 isolates of the *Enterobacteriaceae* (mainly *K. pneumoniae* and *E. coli*), 80 *P.*

TABLE 1 Isolates analyzed in the study

Organism	No. of specimens harboring *bla* genes					
	Total	KPC	NDM	VIM	OXA-48	IMP-1
Enterobacteriaceae	343	83	73[a]	51	89[a]	4
A. baumannii	44	0	5	0	0	20
P. aeruginosa	80	1	0	31	0	16

[a]Nine specimens harbored both bla_{NDM} and bla_{OXA-48}.

aeruginosa isolates, and 44 *A. baumannii* isolates. Details about the strains analyzed are shown in Table 1.

Isolates grown on both BA and MCA were tested with the Xpert Carba-R assay (separate results were generated for each of the agar types), for a total of 934 results. Xpert Carba-R results were available for 99.9% (933/934) of the isolates on the first attempt. One isolate resulted in a no-result outcome upon initial testing but yielded valid results upon repeat testing. Thus, the overall rate of assay success was 100% (934/934).

The Xpert Carba-R assay demonstrated an overall sensitivity of 100% (95% CI, 99.0 to 100%) and an overall specificity of 98.1% (95% CI, 93.1 to 99.8%) relative to the results of reference DNA sequencing of colonies grown on BA (Table 2). The overall result was defined as positive for the Xpert Carba-R assay if any of the targets were positive and negative for the Xpert Carba-R assay if all of the targets were negative. When tested with isolates from MCA, the Xpert Carba-R assay demonstrated an overall sensitivity of 100% (95% CI, 99.0 to 100%) and an overall specificity of 97.1% (95% CI, 91.7 to 99.4%) relative to the results of reference sequencing performed with the isolates from BA (Table 2). For each of the 5 assay targets, the Xpert Carba-R assay, when tested with isolates from BA and MCA, demonstrated a 100% sensitivity and a ≥99.7% specificity compared with the results of reference sequencing (Table 3).

There were 10 isolates for which at least one of the methods detected 2 targets. For 9 samples that were positive for bla_{NDM} and bla_{OXA-48}, the Xpert Carba-R assay results were concordant with the reference sequencing results. One isolate was positive for both the bla_{VIM} and bla_{KPC} targets by the Xpert Carba-R assay (for BA only) but for the bla_{VIM} target only by reference sequencing; for assay performance, the result for the bla_{KPC} target was classified as a false positive.

Discrepant results occurred in 4 samples, as listed below and shown in Table 3.

IMP. The amplification products for one *P. aeruginosa* isolate produced bands by the Agilent bioanalyzer corresponding to the bla_{IMP} target gene, as detected by the Xpert Carba-R assay. The sequencing result for this specimen exhibited 92.95% sequence homology, which was slightly below the 95% cutoff criterion, resulting in a false-positive call for the study analysis.

VIM. Discrepant testing of the one *P. aeruginosa* isolate from MCA produced a 100% sequence match with the bla_{VIM} target, demonstrating concordance between the Xpert Carba-R assay and reference sequencing.

For the false-negative bla_{VIM} result, the clinical site reported that upon resubculture and testing of the isolate, the Xpert Carba-R assay resulted in detection of the bla_{VIM}

TABLE 2 Overall Xpert Carba-R assay (on BA and MCA) results versus reference sequencing (on BA) results for *Enterobacteriaceae*, *A. baumannii*, and *P. aeruginosa*

Target (medium)[a]	No. of specimens with the following result[b]:				Sensitivity (%)	Specificity (%)
	Total	TP	FP	TN	FN	
Overall (BA)	467	364	2	101	0	100 (99.0–100)[c]	98.1 (93.1–99.8)
Overall (MCA)	467	364	3	100	0	100 (99.0–100)	97.1 (91.7–99.4)

[a]Overall refers to the results for the *Enterobacteriaceae*, *A. baumannii*, and *P. aeruginosa*.
[b]TP, true positive; FP, false positive; TM, true negative; FN, false negative.
[c]Values in parentheses are 95% CIs.

TABLE 3 Xpert Carba-R (on BA and MCA) results versus reference sequencing (on BA) results by target for *Enterobacteriaceae*, *A. baumannii*, and *P. aeruginosa*

Target	No. of specimens with the following result[e]:					Sensitivity (%)	Specificity (%)
	Total	TP	FP	TN	FN		
IMP-1	467	40	1[a]	426	0	100 (91.2–100)[f]	99.80 (98.7–100)
VIM	467	82	1[b]	384	0	100 (95.6–100)	99.70 (98.6–100)
NDM	467	78	0/1[c]	389/388	0	100 (95.4–100)	100/99.7 (99.0–100/98.6–100)
KPC	467	84	1[d]/0	382/383	0	100 (95.7–100)	99.7/100 (98.6–100/99.0–100)
OXA-48	467	89	0	378	0	100 (95.9–100)	100 (99.0–100)

[a]The bidirectional DNA sequencing result for this specimen false positive for IMP-1 exhibited 92.95% sequence homology, which was slightly below the 95% cutoff criterion. Discrepant testing was not performed.
[b]By discrepant testing, 1 of 1 specimen was VIM positive.
[c]The clinical site reported that in-house characterization of this false-positive specimen prior to study testing resulted in a positive NDM gene target. Discrepant testing did not produce a sequence match for any of the 5 gene targets.
[d]This false-positive specimen is likely due to KPC cross-contamination at the level of sample preparation. Discrepant testing did not produce a sequence match with the KPC target. Discrepant testing produced a sequence match with the VIM target; therefore, this specimen is classified as true positive in the overall assessment (for *Enterobacteriaceae*, *A. baumannii*, and *P. aeruginosa*) presented in Table 2.
[e]TP, true positive; FP, false positive; TM, true negative; FN, false negative.
[f]Values in parentheses are 95% CIs.

gene target for isolates from both the blood agar and MCA plates. As a result of the resubculture, the Xpert Carba-R assay and reference sequencing results were concordant. As the study protocol did not include resubculturing of specimens, this specimen remained classified as false negative for the primary data analyses presented in this report.

NDM. Discrepant testing of one *K. pneumoniae* isolate from MCA false positive for *bla*$_{NDM}$ did not produce a sequence match with the *bla*$_{NDM}$ target. This false-positive isolate was from the bacterial collection of a center and was previously positive for *bla*$_{NDM}$ using an in-house molecular method (data not shown).

KPC. The false-positive results for the *K. pneumoniae* isolates were likely due to *bla*$_{KPC}$ cross-contamination at the level of sample preparation, as four other *bla*$_{KPC}$-positive isolates were processed and tested with the Xpert Carba-R assay on the same day. Discrepant testing of the MCA isolate from the false-positive specimen did not produce a sequence match with the *bla*$_{KPC}$ target.

DISCUSSION

Detection of CROs is a critical issue for clinical laboratories to provide guidance for infection control activities and potentially for the targeted therapy (21). The Xpert Carba-R assay can be used directly on rectal swab specimens and provides highly reliable results in less than 1 h from the time of specimen collection (9, 20). The assay detects *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$, including the *bla*$_{OXA-48-like}$ variants (*bla*$_{OXA-181}$ and *bla*$_{OXA-232}$), in *Enterobacteriaceae*, *P. aeruginosa*, and *A. baumannii*, with a significant impact on the rapid identification of patients with gastrointestinal colonization with CROs (9, 20).

In the present study, we tested the Xpert Carba-R assay on bacterial isolates from both a Gram-negative bacterium-selective medium (MCA) and a nonselective medium (BA), in order to evaluate the specificity and sensitivity of the method for detecting the five most common families of carbapenemase genes. The test showed excellent performance both from BA and from MCA in our multicenter study, as also documented in other assessments performed in single institutions (22, 23).

Possible application of the Xpert Carba-R assay on bacterial isolates could be as an ancillary test for carbapenem-nonsusceptible Gram-negative microorganisms from blood cultures, urine, intra-abdominal, or lower-respiratory-tract specimens to determine if they are carbapenemase producers. The confirmation of the results through a molecular assay guides infection prevention activities, such as the use of contact precautions or cohorting of patients with similar infections, and potentially therapeutic

strategies. The correct identification of carbapenemase genes is also necessary to address therapeutic options using the newer beta-lactam–beta-lactamase inhibitors with activity against serine-based carbapenemases (e.g., avibactam has no activity against MBLs, varborbactam covers only KPCs, and relebactam may have a different degree of efficacy related to the presence of particular genes) (24). Moreover, molecular confirmation of carbapenemase gene carriage may be useful to correctly identify CROs expressing low MICs for carbapenems, as sometimes happens for bla_{VIM} and bla_{OXA-48} (25–27).

The use of the Xpert Carba-R assay could be indicated subsequent to traditional antimicrobial susceptibility testing to evaluate Gram-negative microorganisms isolated from critical clinical samples (e.g., blood samples) in patients who are known to be at risk of developing infections caused by CROs, such as those known to be intestinal carriers. Since colonization often precedes infection, it is likely that the colonizing CRO organism may later cause clinical infections (27, 28). This molecular approach has the advantages, compared with other phenotypic methods, such as the Carba NP assay or the CIM, of being quicker and being able to identify the resistance gene involved, allowing a prompt start of an adequate therapy.

The currently available version of the Xpert Carba-R assay enables the detection of several emerging $bla_{OXA-48-like}$ genes (i.e., $bla_{OXA-181}$ and $bla_{OXA-232}$) (29). In areas where these mechanisms of resistance are emerging, a rapid method of detecting them can be important for infection prevention activities. If it is not possible to perform molecular analysis directly on rectal swabs (for either economic or practical reasons), the test could be performed on specimens that show the growth of putative colonies at 24 h to 48 h of incubation, prior to phenotypic confirmatory tests. Patients with positive results could be isolated 24 h earlier, which would provide unquestionable benefits in term of the prevention of spreading. In addition, the Xpert Carba-R assay allows the exact determination of the gene family responsible for resistance, information not always provided by phenotypic tests (30). This is important for MBL genes, which are indistinguishable in DDST. Correct knowledge of the genes involved is of great importance in tracing and analyzing the origin and diffusion of local outbreaks.

The screening of patients for CRO colonization or the confirmation of CRO infection may use a combination of genotypic and phenotypic approaches, depending on the local epidemiology, the prevalence rates of CRO colonization and infection, economic and technical resources, and infection control policies.

This study was performed in four different institutions without experiencing particular challenges in validating the assay. The main problem was the analysis of bla_{IMP} isolates, since the epidemiology of the different centers allowed the evaluation of only one clinical isolate. The remaining 39 specimens which were positive for bla_{IMP} were from contrived samples which contained bla_{IMP-1}, bla_{IMP-6}, and bla_{IMP-10}. The assay identifies and reports the results for bla_{IMP-1}, but it is able to detect also bla_{IMP-2}, bla_{IMP-4}, bla_{IMP-6}, bla_{IMP-10}, and bla_{IMP-11}. On the contrary, bla_{IMP-7}, bla_{IMP-13}, and bla_{IMP-14} are not detectable. The false-positive bla_{IMP} found in our study showed 92.95% sequence homology, slightly below the 95% threshold criterion set to define positivity. It was not possible to correctly identify the gene, since it was not sequenced entirely, but it is highly likely that it was related to the variants detected through the assay.

The Xpert Carba-R assay also has some limitations. Several carbapenemase genes, as stated above, were not included among the targets detected. Other examples include the chromosomally encoded bla_{SME} genes, which may be expressed in some strains of *Serratia marcescens* (31), and the most common plasmid-encoded carbapenem resistance genes from *A. baumannii*, bla_{OXA-23} and bla_{OXA-58}. Moreover, other mechanisms of carbapenem resistance not mediated by the production of carbapenemases cannot be detected. Finally, the ability of bacterial species to mutate and select new allelic variants of previously known resistance genes or to acquire new resistance determinants could, in the future, be related to false-negative results by using molecular tests. Considering the possible limitations mentioned above, data obtained from the Xpert Carba-R assay should be considered predictive of carbapenem resistance if they are positive for the targets evaluated, whereas the negative results should always be

confirmed through phenotypic confirmatory tests before excluding the possibility that the isolate identified is a CRO.

ACKNOWLEDGMENTS

REFERENCES

1. Canton R, Akova M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Miriagou V, Naas T, Rossolini GM, Samuelsen O, Seifert H, Woodford N, Nordmann P, European Network on Carbapenemases. 2012. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. Clin Microbiol Infect 18:413–431. https://doi.org/10.1111/j.1469-0691.2012.03821.x.

2. Centers for Disease Control and Prevention. 2013. Antibiotic resistance threats in the United States, 2013. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/drugresistance/threat-report-2013.

3. Falagas ME, Tansarli GS, Karageorgopoulos DE, Vardakas KZ. 2014. Deaths attributable to carbapenem-resistant Enterobacteriaceae infections. Emerg Infect Dis 20:1170–1175. https://doi.org/10.3201/eid2007.121004.

4. Kitchel B, Rasheed JK, Patel JB, Srinivasan A, Navon-Venezia S, Carmeli Y, Brolund A, Giske CG. 2009. Molecular epidemiology of KPC-producing Klebsiella pneumoniae isolates in the United States: clonal expansion of multilocus sequence type 258. Antimicrob Agents Chemother 53:3365–3370. https://doi.org/10.1128/AAC.00126-09.

5. Adler A, Hussein O, Ben-David D, Masarwa S, Navon-Venezia S, Schwaber MJ, Carmeli Y, Post-Acute-Care Hospital Carbapenem-Resistant Enterobacteriaceae Working Group. 2015. Persistence of Klebsiella pneumoniae ST258 as the predominant clone of carbapenemase-producing Enterobacteriaceae in post-acute-care hospitals in Israel, 2008-13. J Antimicrob Chemother 70:89–92. https://doi.org/10.1093/jac/dku333.

6. Gaiarsa S, Comandatore F, Gaibani P, Corbella M, Dalla Valle C, Epis S, Scaltriti E, Carretto E, Farina C, Labonia M, Landini MP, Pongolini S, Sambri V, Bandi C, Marone P, Sassera D. 2015. Genomic epidemiology of Klebsiella pneumoniae in Italy and novel insights into the origin and global evolution of its resistance to carbapenem antibiotics. Antimicrob Agents Chemother 59:389–396. https://doi.org/10.1128/AAC.04224-14.

7. Poirel L, Hombrouck-Alet C, Freneaux C, Bernabeu S, Nordmann P. 2010. Global spread of New Delhi metallo-beta-lactamase 1. Lancet Infect Dis 10:832. https://doi.org/10.1016/S1473-3099(10)70279-6.

8. Cornaglia G, Giamarellou H, Rossolini GM. 2011. Metallo-beta-lactamases: a last frontier for beta-lactams? Lancet Infect Dis 11:381–393. https://doi.org/10.1016/S1473-3099(11)70056-1.

9. Moore NM, Cantón R, Carretto E, Peterson LR, Sautter RL, Traczewski MM, the Carba-R Study Team. 2017. Rapid identification of five classes of carbapenem resistance genes directly from rectal swabs by use of the XPERT Carba-R assay. J Clin Microbiol 55:2268–2275. https://doi.org/10.1128/JCM.00137-17.

10. Oueslati S, Nordmann P, Poirel L. 2015. Heterogeneous hydrolytic features for OXA-48-like beta-lactamases. J Antimicrob Chemother 70:1059–1063. https://doi.org/10.1093/jac/dku524.

11. Potron A, Poirel L, Nordmann P. 2014. Derepressed transfer properties leading to the efficient spread of the plasmid encoding carbapenemase OXA-48. Antimicrob Agents Chemother 58:467–471. https://doi.org/10.1128/AAC.01344-13.

12. Towner KJ. 2009. Acinetobacter: an old friend, but a new enemy. J Hosp Infect 73:355–363. https://doi.org/10.1016/j.jhin.2009.03.032.

13. Centers for Disease Control and Prevention. 2015. Guidance for control of carbapenem-resistant Enterobacteriaceae (CRE). Toolkit. Centers for Disease Control and Prevention, Atlanta, GA.

14. Public Health England. 2013. Acute trust toolkit for the early detection, management and control of carbapenemase-producing Enterobacteriaceae. Public Health England, London, United Kingdom.

15. Clinical and Laboratory Standards Institute. 2016. Performance standards for antimicrobial susceptibility testing, 26th ed. M100S. Clinical and Laboratory Standards Institute, Wayne, PA.

16. Nordmann P, Poirel L, Dortet L. 2012. Rapid detection of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis 18:1503–1507. https://doi.org/10.3201/eid1809.120355.

17. van der Zwaluw K, de Haan A, Pluister GN, Bootsma HJ, de Neeling AJ, Schouls LM. 2015. The carbapenem inactivation method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in gram-negative rods. PLoS One 10:e0123690. https://doi.org/10.1371/journal.pone.0123690.

18. Doyle D, Peirano G, Lascols C, Lloyd T, Church DL, Pitout JD. 2012. Laboratory detection of Enterobacteriaceae that produce carbapenemases. J Clin Microbiol 50:3877–3880. https://doi.org/10.1128/JCM.02117-12.

19. Morey KE, Vega R, Cassidy PM, Buser GL, Rayar JK, Myers JA, Weissman SJ, Beldavs ZG, Pfeiffer CD. 2017. Evaluation of the Carba NP test in Oregon, 2013. Antimicrob Agents Chemother 61:e03005-15. https://doi.org/10.1128/AAC.03005-15.

20. Tato M, Ruiz-Garbajosa P, Traczewski M, Dodgson A, McEwan A, Humphries R, Hindler J, Veltman J, Wang H, Canton R. 2016. Multisite evaluation of Cepheid Xpert Carba-R assay for detection of carbapenemase-producing organisms in rectal swabs. J Clin Microbiol 54:1814–1819. https://doi.org/10.1128/JCM.00341-16.

21. Iovleva A, Doi Y. 2017. Carbapenem-resistant Enterobacteriaceae. Clin Lab Med 37:303–315. https://doi.org/10.1016/j.cll.2017.01.005.

22. Smith M, Diederen B, Scharringa J, Leversteijn-van Hall M, Fluit AC, Cohen Stuart J. 2016. Rapid and accurate detection of carbapenemase genes in Enterobacteriaceae with the Cepheid Xpert Carba-R assay. J Med Microbiol 65:951–953. https://doi.org/10.1099/jmm.0.000310.

23. Miller SA, Hindler JA, Chengcuenca A, Humphries RM. 2017. Use of ancillary carbapenemase tests to improve specificity of phenotypic definitions for carbapenemase producing Enterobacteriaceae. J Clin Microbiol 55:1827–1836. https://doi.org/10.1128/JCM.00157-17.

24. Zhanel GG, Lawson CD, Adam H, Schweizer F, Zelenitsky S, Lagace-Wiens PR, Denisuik A, Rubinstein E, Gin AS, Hoban DJ, Lynch JP, III, Karlowsky JA. 2013. Ceftazidime-avibactam: a novel cephalosporin/beta-lactamase inhibitor combination. Drugs 73:159–177. https://doi.org/10.1007/s40265-013-0013-7.

25. Poirel L, Potron A, Nordmann P. 2012. OXA-48-like carbapenemases: the phantom menace. J Antimicrob Chemother 67:1597–1606. https://doi.org/10.1093/jac/dks121.

26. Tato M, Morosini M, Garcia L, Alberti S, Coque MT, Canton R. 2010. Carbapenem heteroresistance in VIM-1-producing Klebsiella pneumoniae isolates belonging to the same clone: consequences for routine suscep-

tibility testing. J Clin Microbiol 48:4089–4093. https://doi.org/10.1128/JCM.01130-10.

27. Barbarini D, Russello G, Brovarone F, Capatti C, Colla R, Perilli M, Moro ML, Carretto E. 2015. Evaluation of carbapenem-resistant *Enterobacteriaceae* in an Italian setting: report from the trench. Infect Genet Evol 30:8–14. https://doi.org/10.1016/j.meegid.2014.11.025.

28. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. 2012. Carbapenemases in *Klebsiella pneumoniae* and other *Enterobacteriaceae*: an evolving crisis of global dimensions. Clin Microbiol Rev 25:682–707. https://doi.org/10.1128/CMR.05035-11.

29. Dortet L, Fusaro M, Naas T. 2016. Improvement of the Xpert Carba-R kit for the detection of carbapenemase-producing *Enterobacteriaceae*. Antimicrob Agents Chemother 60:3832–3837. https://doi.org/10.1128/AAC.00517-16.

30. Aguirre-Quinonero A, Martinez-Martinez L. 2017. Non-molecular detection of carbapenemases in *Enterobacteriaceae* clinical isolates. J Infect Chemother 23:1–11. https://doi.org/10.1016/j.jiac.2016.09.008.

31. Pollett S, Miller S, Hindler J, Uslan D, Carvalho M, Humphries RM. 2014. Phenotypic and molecular characteristics of carbapenem-resistant *Enterobacteriaceae* in a health care system in Los Angeles, California, from 2011 to 2013. J Clin Microbiol 52:4003–4009. https://doi.org/10.1128/JCM.01397-14.