Evaluation of PCR-Based Testing for Surveillance of KPC-Producing Carbapenem-Resistant Members of the Enterobacteriaceae Family

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The spread of carbapenem-resistant members of the Enterobacteriaceae family (CRE) harboring carbapenemases is an emerging public health threat. As KPC-producing Klebsiella species are endemic in our tertiary care hospital, we aimed to evaluate a PCR-based surveillance test for identification of rectal carriage of KPC-producing CRE. We conducted a surveillance study between May and December 2007. Rectal swabs were collected from patients known to harbor CRE and from contacts of newly discovered patients harboring CRE. Specimens were evaluated by culture and by PCR analysis for blaKPC and were defined as positive if CRE was cultured and blaKPC was identified. Discrepant results between the culture and PCR analysis were resolved by subculturing, repeating the PCR, and performing a hydrolysis assay. Positive CRE cultures prior to or subsequent to the time of sampling for the study were also taken into consideration. Sensitivity, specificity, and time to result were calculated. A total of 755 swabs were included. Concordant results were documented for 735 specimens; 51 were positive as determined by both PCR and culture. Discrepancies existed for 20 swabs; 9 were blaKPC negative and CRE culture positive, and 11 were blaKPC positive and CRE culture negative. After repeat testing, a total of 64 samples were classified as blaKPC-positive CRE. The sensitivity and specificity of the PCR analysis were 92.2% and 99.6%, respectively, and those of the culture were 87.5% and 99.4%, respectively. Over the last 3 months of the study, the sensitivity of the PCR improved to 96.3%, versus 77.8% for culture. Time to result was 30 h for the PCR and 60 h (negative) and 75 h (positive) for the CRE culture. blaKPC PCR-based testing is a useful method for the surveillance of KPC-producing CRE. Its main advantage over culturing is a shorter time to result, and it may prove to be more sensitive.

KPC-mediated carbapenem resistance in members of the Enterobacteriaceae has emerged recently in Israel, as observed in clinical strains of Escherichia coli (14), Enterobacter cloacae (13), and Klebsiella pneumoniae (10). An epidemic clone of KPC-3-producing Klebsiella pneumoniae, possessing resistance to nearly all antimicrobial agents, excluding gentamicin and colistin, has spread in all major Israeli hospitals (15). Infections caused by this strain have been associated with greater than 40% in-hospital mortality (23). As part of our extensive infection control efforts to limit the spread of this highly epidemic strain in the hospital, we aimed to implement a policy of early detection of carriers by screening for gastrointestinal carriage of this pathogen.

Active surveillance has been shown to be an effective infection control strategy with other antibiotic-resistant pathogens, such as methicillin (meticillin)-resistant Staphylococcus aureus (4, 5) and vancomycin-resistant enterococci (16). The use of culture-based methods for detection of carbapenem-resistant members of the Enterobacteriaceae family (CRE) from rectal or stool surveillance samples was evaluated previously, using selective and nonselective broth media supplemented with an imipenem disk, followed by plating on MacConkey agar with and without imipenem (9). PCR-based assays were also assessed for detection of carbapenem-resistant K. pneumoniae from rectal surveillance swabs (6, 8). Although good performance was shown by both methods, there is still no recommended reference method for the recovery of CRE in the context of active surveillance. Moreover, automated susceptibility testing systems may fail to detect KPC-mediated resistance in clinical isolates (1, 24).

We aimed to implement a rapid test for identification of CRE gastrointestinal carriage based on a molecular method. Since in our institution carbapenem resistance in members of the Enterobacteriaceae, and specifically in the epidemic K. pneumoniae strain, is rendered by the presence of blaKPC, we designed and evaluated a PCR-based method for blaKPC detection in rectal specimens. We compared the performance of this newly developed molecular-based diagnostic tool with that of a culture-based method for identifying carriers of KPC-producing CRE.

(This work was presented in part at the 48th Interscience Conference on Antimicrobial Agents and Chemotherapy/46th Annual Meeting of the Infectious Diseases Society of America, Washington, DC, 25 to 28 October 2008 [20].)

MATERIALS AND METHODS

Site, patient selection, and collection of surveillance specimens. We conducted a surveillance study at the Tel-Aviv Sourasky Medical Center, a 1,200-bed tertiary care hospital in Tel-Aviv, Israel, from 22 May through 31 December 2007. Rectal specimens were collected from known CRE carriers and from contacts of
patients newly discovered to be harboring CRE. A positive CRE isolate was defined by a MIC of $\geq$16 g/ml for either imipenem or meropenem using the Vitek 2 automated system (bioMerieux, Marcy-L’Etoile, France) and Etest for validation (AB Biodisk, Solna, Sweden). Enrollment criteria for known CRE carriers included (i) isolation of CRE from a clinical sample or (ii) readmission of a patient with a prior isolation of CRE from either a clinical sample or a surveillance specimen. Enrollment criteria for contacts of patients known to harbor CRE included all of the following: (i) a length of hospitalization greater than 24 h in the same ward with a newly identified patient harboring CRE, by either a clinical sample or a surveillance specimen; (ii) treatment by the same nursing staff; and (iii) hospitalization at the time of survey. Rectal specimens were collected, and swabs were transferred immediately to the laboratory. The work plan is detailed in Fig. 1.

Culture-based method for CRE detection. Swabs were streaked directly onto MacConkey agar plates supplemented with 1 g/ml imipenem (PD-032; Hy-Labs, Rehovot, Israel). Each swab was plated by being rolled several times onto the agar plate and then enriched in brain heart infusion broth containing 8 g/ml ertapenem (until 21 June 2007) or in nonselective brain heart infusion broth (from 22 June 2007 and onward). Imipenem-containing plates and broths were incubated at 37°C overnight (18 to 24 h) before further processing, and plates were further incubated for an additional 24 h to allow for late growth. Colonies obtained on the imipenem-containing MacConkey plates that were visualized macroscopically as members of the Enterobacteriaceae were evaluated by Vitek 2 using the AST-GN09 card for identification and susceptibility testing. Subculturings onto fresh imipenem-containing MacConkey agar plates was performed when needed. Colonies suspected as nonfermenters were transferred into Enterotubes (Hy-Enterotest TT-146; Hy-Labs, Rehovot, Israel) for identification and were tested for the presence of oxidase (oxidase strips; Novamed, Jerusalem, Israel). Colonies that turned out to be fermenters were identified to the species level using Vitek 2. After primary analysis, all bacteria identified as CRE by Vitek 2 and inoculated broths were stored at $-80^\circ$C for further analysis if necessary. MIC testing of imipenem and meropenem was verified by Etest (AB Biodisk, Solna, Sweden).

Molecular-based method for KPC-producing CRE detection. Molecular identification of KPC-producing CRE was performed by blaKPC PCRs using bacterial lysates obtained from swab-inoculated broths and from colonies suspected as being CRE obtained on imipenem-containing MacConkey plates. Bacterial cell lysates from overnight broths were prepared by removal of 200 μl of broth culture, centrifugation (12,000 × g; 2 min), resuspension in 200 μl of molecular-grade water (Bio-Lab, Israel), boiling at 95°C for 10 min, and discarding the cellular debris by centrifugation (12,000 × g; 2 min at 4°C). Bacterial lysates derived from colonies suspected to be CRE were prepared by inoculating a single colony into 100 μl of molecular-grade water and then prepared as detailed above. PCR analysis for blaKPC was performed with 1 μl of cell lysates, using the following primers designed to identify all blaKPC genes (blaKPC-1 through blaKPC-7): KPC forward (ATGTCACTGTATCGCCGTCT) and KPC reverse (TTTTCAGAGCCTTTACTGCCC) (13). Lysates derived from Escherichia coli ATCC 25922 and blaKPC-carrying K. pneumoniae strain 490 (10) were used as negative and positive controls, respectively, in each PCR. The PCR conditions were as follows: 15 min at 95°C and 38 cycles of 1 min at 94°C, 1 min at 62°C, and 1 min at 72°C, followed by an extension step of 10 min at 72°C. PCRs were performed with Hot-Star Taq DNA polymerase (Qiagen, Hilden, Germany), and the resulting PCR products were analyzed in a 1% agarose gel with ethidium bromide staining and UV light.

Analysis of discrepant results. All swabs yielding discrepant results between the two testing methods regarding the presence of KPC-producing CRE in the primary analysis were subjected to a second analysis (Fig. 1). For samples with negative results determined by PCR and a positive CRE culture in the primary analysis, a blaKPC PCR assay was repeated on a broth
negative predictive value (NPV), and time to positive and negative result of the positive, the microbiological test result was deemed to be false negative. If the second PCR test was deemed to be false positive; if the second PCR test was negative, a modified Hodge test was performed on the CRE isolate (stored at 80°C), using the test described previously by Lee et al. (11). Isolates with a positive Hodge test were considered non-carbapenemase-producing CRE, and the culture was deemed false positive (for identification of KPC-producing CRE). Of the 11 non-KPC-producing CRE, 80 specimens showed discrepant results between the PCR and culture methods (Table 1). Nine specimens grew CRE (all isolates were K. pneumoniae) but were bla<sub>KPC</sub> negative, and 11 specimens did not grow CRE but were bla<sub>KPC</sub> positive. Further laboratory analysis was completed with these discrepant samples according to the study work plan (Fig. 1).

Five of the nine specimens that grew CRE but were bla<sub>KPC</sub> negative at first yielded a positive PCR result when retested and were interpreted as false negative by PCR. The remaining four specimens were confirmed as bla<sub>KPC</sub> negative upon retesting and were also determined to be negative by the modified Hodge test, thus finally being interpreted as containing non-KPC-producing CRE. Of the 11 bla<sub>KPC</sub>-positive specimens from which CRE did not grow on the selective MacConkey plate, 8 were classified as containing KPC-producing CRE; of these, 4 yielded a CRE microorganism by subculture of the broth (3 isolates were K. pneumoniae and 1 was Enterobacter cloacae), 3 were taken from confirmed carriers of CRE, and in 1 case CRE grew following repeat perirectal sampling (1 week after the initial sample was taken). As the screened patients were contacts of known CRE-positive patients, it is possible that this second culture represented newly acquired colonization versus a false-negative initial culture. An additional three specimens were found to be bla<sub>KPC</sub> negative by

**RESULTS**

A total of 755 rectal swabs, collected consecutively from 650 patients between 22 May 2007 and 31 December 2007, were included for analysis. Thirty-nine swabs were taken from patients with a previous positive CRE culture (three of whom had a prior surveillance culture positive for CRE), and 716 swabs were collected from hospitalized patients who had contact with a CRE carrier. All specimens underwent both microbiological and molecular processing. After primary analysis, 60 specimens were positive based on culture and 62 were positive based on PCR.

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**TABLE 1. Laboratory analysis of 20 discrepant results and final interpretation**

<table>
<thead>
<tr>
<th>Swab no.</th>
<th>Result from first analysis&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Result from second analysis&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Final interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>586</td>
<td>CRKP</td>
<td>CRE negative</td>
<td>CRE negative</td>
</tr>
<tr>
<td>224</td>
<td>CRKP</td>
<td>NA</td>
<td>KPC-positive CRE</td>
</tr>
<tr>
<td>208</td>
<td>CRKP</td>
<td>NA</td>
<td>KPC-negative CRE</td>
</tr>
<tr>
<td>411</td>
<td>CRKP</td>
<td>NA</td>
<td>KPC-positive CRE</td>
</tr>
<tr>
<td>413</td>
<td>CRKP</td>
<td>NA</td>
<td>KPC-positive CRE</td>
</tr>
<tr>
<td>336</td>
<td>CRKP</td>
<td>NA</td>
<td>KPC-positive CRE</td>
</tr>
<tr>
<td>675</td>
<td>CRKP</td>
<td>NA</td>
<td>KPC-negative CRE</td>
</tr>
<tr>
<td>979</td>
<td>CRKP</td>
<td>NA</td>
<td>KPC-positive CRE</td>
</tr>
<tr>
<td>533</td>
<td>CRKP</td>
<td>NA</td>
<td>KPC-positive CRE</td>
</tr>
<tr>
<td>766</td>
<td>CRE negative</td>
<td>CRE negative</td>
<td>CRE negative</td>
</tr>
<tr>
<td>382</td>
<td>CRE negative</td>
<td>NA</td>
<td>KPC-positive CRE</td>
</tr>
<tr>
<td>972</td>
<td>CRE negative</td>
<td>NA</td>
<td>KPC-positive CRE&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>987</td>
<td>CRE negative</td>
<td>NA</td>
<td>KPC-positive CRE&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>970</td>
<td>CRE negative</td>
<td>NA</td>
<td>KPC-positive CRE&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>757</td>
<td>CRE negative</td>
<td>NA</td>
<td>CRE negative</td>
</tr>
<tr>
<td>864</td>
<td>CRE negative</td>
<td>CRENT</td>
<td>KPC-positive CRE</td>
</tr>
<tr>
<td>592</td>
<td>CRE negative</td>
<td>NA</td>
<td>CRE negative</td>
</tr>
<tr>
<td>628</td>
<td>CRE negative</td>
<td>CRKP</td>
<td>KPC-positive CRE</td>
</tr>
<tr>
<td>873</td>
<td>CRE negative</td>
<td>NA</td>
<td>KPC-positive CRE&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>428</td>
<td>CRE negative</td>
<td>CRKP</td>
<td>KPC-positive CRE</td>
</tr>
</tbody>
</table>

<sup>a</sup> CRKP, carbapenem-resistant Klebsiella pneumoniae; CRENT, carbapenem-resistant Enterobacter cloacae; NA, not available; +, a positive bla<sub>KPC</sub> PCR result; −, a negative bla<sub>KPC</sub> PCR result.

<sup>b</sup> First detection directly from swab (as detailed in Materials and Methods).

<sup>c</sup> Results of retesting from thawed swab-cultured broths (as detailed in Materials and Methods).

<sup>d</sup> A prior positive clinical culture of CRKP.

<sup>e</sup> Another specimen taken by a rectal swab 1 week later was positive for CRKP and bla<sub>KPC</sub>.

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**Concordant results.** Concordant results between microbiological CRE testing and molecular bla<sub>KPC</sub> testing were documented for 735 specimens; of these, 51 swabs (6.9%) tested positive by both methods and 684 (93.1%) tested negative. Of the 51 CRE isolates recovered from the 51 swabs, 50 isolates were identified as K. pneumoniae and 1 isolate was identified as Enterobacter cloacae.

**Discrepant results.** Overall, 20 specimens showed discrepant results between the PCR and culture methods (Table 1). Nine specimens grew CRE (all isolates were K. pneumoniae) but were bla<sub>KPC</sub> negative, and 11 specimens did not grow CRE but were bla<sub>KPC</sub> positive. Further laboratory analysis was completed with these discrepant samples according to the study work plan (Fig. 1).

Five of the nine specimens that grew CRE but were bla<sub>KPC</sub> negative at first yielded a positive PCR result when retested and were interpreted as false negative by PCR. The remaining four specimens were confirmed as bla<sub>KPC</sub> negative upon retesting and were also determined to be negative by the modified Hodge test, thus finally being interpreted as containing non-KPC-producing CRE. Of the 11 bla<sub>KPC</sub>-positive specimens from which CRE did not grow on the selective MacConkey plate, 8 were classified as containing KPC-producing CRE; of these, 4 yielded a CRE microorganism by subculture of the broth (3 isolates were K. pneumoniae and 1 was Enterobacter cloacae), 3 were taken from confirmed carriers of CRE, and in 1 case CRE grew following repeat perirectal sampling (1 week after the initial sample was taken). As the screened patients were contacts of known CRE-positive patients, it is possible that this second culture represented newly acquired colonization versus a false-negative initial culture. An additional three specimens were found to be bla<sub>KPC</sub> negative by
Table 2. Comparison of culture-based identification and PCR-based identification of blaKPC-producing CRE after second analysis (755 rectal specimens)

<table>
<thead>
<tr>
<th></th>
<th>Microbiological identification</th>
<th>Molecular identification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>56</td>
<td>8</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>687</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>695</td>
</tr>
</tbody>
</table>

*Sensitivity, 92.2%; specificity, 99.6%; PPV, 99.6%; NPV, 98.8%.
*b Sensitivity, 92.2%; specificity, 99.6%; PPV, 95.2%; NPV, 99.3%.

repeat PCR testing and thus were ultimately interpreted as CRE negative, i.e., yielding a false-positive primary PCR assay (Table 1).

A summary of the performance of blaKPC PCR testing, including sensitivity, specificity, PPV, and NPV upon retesting after secondary analysis, is presented in Table 2. Detection by PCR improved over time, and for the last 3 months of the study (257 swabs taken between 1 October and 31 December 2007), sensitivities and specificities were 96.3% and 100% for the PCR-based assay and 77.8% and 100% for the culture-based assay, respectively ($P = 0.011$ for sensitivities).

Time to both a positive and a negative result was significantly shorter for the PCR-based assay than for the culture: 30 h versus 60 h for a negative result ($P < 0.001$), and 30 h versus 75 h for a positive result ($P < 0.001$).

**DISCUSSION**

Carbapenem resistance in members of the *Enterobacteriaceae* is mediated by either a combination of a beta-lactamase (such as ESBL or AmpC) with impermeability (3, 7) or by the production of a carbapenemase (18). While the former mechanism occurs occasionally among broad-spectrum beta-lactamase producers, it does not seem so far to have epidemic potential, probably due to the reduced fitness of organisms that lose their major porin. On the other hand, enteric strains harboring carbapenemases, which are plasmid-encoded enzymes, show remarkable epidemic success (22). The rapid spread of epidemic clones of KPC-producing members of the *Enterobacteriaceae* within a geographical area has been reported from New York City hospitals (2, 25) and also from Israeli hospitals during 2006–2007 (mainly *K. pneumoniae* but also *E. coli*, *Enterobacter* species, and other members of the *Enterobacteriaceae*) (10, 13–15, 19). These organisms are almost always extensively drug-resistant strains, toward which very few antibiotic agents are active in vitro, and clinical data on their efficacy are lacking. Thus, the spread of carbapenemases is a threatening clinical and public health problem.

Limiting the spread of epidemic multidrug-resistant members of the *Enterobacteriaceae* inside hospitals requires early detection of gastrointestinal carriers by screening as an integral component of successful infection control strategies (12, 17). Placing fecal surveillance specimens into 5 ml of broth containing a 10-µg imipenem disc was suggested by Landman et al. as an easy screening method for carbapenem-resistant *K. pneumoniae* (9). We aimed to implement a rapid identification method, targeted for gastrointestinal carriage of KPC-producing CRE based on molecular detection of the *blaKPC* gene, and to evaluate its performance compared to culture-based methods. Since there is no gold standard for determining carriage status, we considered a true carrier to be any patient who had a CRE culture-positive specimen with an identified KPC gene or carbapenemase activity.

We tested 755 perirectal specimens for carriage of carbapenemase-producing CRE, of which 64 were interpreted as positive for blaKPC. Analysis of the results showed a high degree of concordance between PCR analysis and the culture-based assay (97% concordance). Apart from the similarly high diagnostic capability of the two methods for detection of CRE carriage, this degree of concordance clearly reflects the predominance of the KPC-producing clone of *K. pneumoniae* among the CRE in our hospital. Likewise, we did not find any other carbapenemase activity by the hydrolysis assay for the CRE culture-positive, *blaKPC*-negative isolates.

Performance of the PCR assay improved over time, and during the last 3 months of the study period only 1 swab (out of 257 taken during that time) was interpreted incorrectly (false negative) by the PCR assay. Since no changes in laboratory processing were documented during this period, we hypothesize that the acquired experience with the molecular technique during the course of the study may have been responsible for the noted trend. Thus, our findings suggest that the PCR is a reliable assay for identifying rectal carriage of KPC-producing CRE. A recently published study using real-time quantitative PCR for the detection of CRE from rectal swabs showed similar performances but required costly steps, including DNA extraction from swabs and highly sophisticated equipment (6).

In settings where a regular PCR machine is used, the relative costs of molecular-based and culture-based methods are similar.

The main advantage of the molecular method over the reference microbiological procedure was a shorter time to result. Using the molecular method, all results (either positive or negative) were available within 30 h, while with the culture-based methods results were obtained within up to 192 h (negative results) and 144 h (positive results). This lag time in culture-based results is due to differences in the number of species and colony morphologies growing on the selective screening plate. The implications of even a 1-day delay in identification of CRE carriers may be vast, since delayed implementation of enhanced infection control measures may result in a large outbreak (21).

Using a PCR-based method for CRE surveillance has several disadvantages: first, the species carrying the *blaKPC* gene and the phenotype remain unknown, i.e., susceptibilities to clinically relevant antibiotics such as the polymyxins and tetracyclines are not tested. This may lead to a delay in identifying epidemiologically important clusters or the emergence of new phenotypes or the spread of *blaKPC* to previously unaffected species. Also, the direction of empirical therapy in the event that a colonized patient exhibits signs of sepsis may be hindered. Second, the method detects only *blaKPC*; thus, in settings where other carbapenemases are prevalent other methods should be used. Third, for the reasons mentioned above,
the emergence of a new mechanism of carbapenem resistance may be missed by this method. In regions where screening of additional carbapenemases is warranted, a wider PCR screening may be implemented using multiple primers designed for the identification of various carbapenemase genes. Additionally, the PCR-based approach may not be applicable as a screening tool in resource-limited centers due to limited availability of both qualified laboratory personnel and the equipment necessary to perform molecular testing.

Our study has several limitations. First, the lack of a gold standard allowed only an internal comparison of the methods studied, and true sensitivity may therefore be lower than reported. Second, we interpreted \( \text{bla}_\text{KPC} \)-positive, culture-negative surveillance specimens as truly positive if they were taken from known CRE carriers, although data concerning the duration of CRE carriage are limited. Finally, our study included patients at high risk for CRE carriage: >90% of the specimens were taken from contacts of CRE carriers, and the study was conducted during an outbreak of KPC-producing \( K \). pneumoniae. Therefore, the generalizability of our results may be limited in non-outbreak settings.

We conclude that \( \text{bla}_\text{KPC} \) PCR-based testing has excellent performance for screening patients for rectal carriage of KPC-producing CRE and is highly applicable for active surveillance. Its main advantage over culture-based methods is a shorter time to result. Due to the discussed limitations, despite the excess cost and labor involved in employing both methodological suggestions, we use this method in conjunction with culture-based methods.

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