Evaluation of Techniques for Detection of Carbapenem-Resistant
\textit{Klebsiella pneumoniae} in Stool Surveillance Cultures

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Screening for gastrointestinal colonization with multidrug-resistant nosocomial pathogens is an important component of infection control protocols. In the New York City region, carbapenem-resistant \textit{Klebsiella pneumoniae} strains, which harbor the \textit{KPC} carbapenem-hydrolyzing \textit{\beta}-lactamase, have rapidly emerged. The potential utility of screening medium, which involved using 10-\(\mu\)g imipenem disks, was investigated. The method of placing a sample from a fecal surveillance culture into broth containing an imipenem disk appeared to have the greatest sensitivity for detecting \textit{K. pneumoniae}. Gastrointestinal colonization with two other carbapenem-resistant nosocomial pathogens, \textit{Pseudomonas aeruginosa} and \textit{Acinetobacter baumannii}, was also detected using this method. Placing fecal surveillance specimens into broth containing an imipenem disk is an easy method for screening samples for carbapenem-resistant nosocomial pathogens.

Controlling the spread of antibiotic-resistant nosocomial pathogens involves a combination of antibiotic control measures and effective infection control strategies. For example, recommendations for the control of vancomycin-resistant enterococci include reduction of vancomycin use, strict adherence to infection control practices, and early detection by screening for gastrointestinal colonization (5). Implementation of these measures has successfully controlled the spread of this pathogen (14). The availability of commercially prepared media that can be used to screen rectal cultures for vancomycin-resistant enterococci facilitates laboratory detection. Similarly, effective control strategies for controlling members of the family \textit{Enterobacteriaceae} with extended-spectrum \textit{\beta}-lactamas (ESBLs) have involved aggressive infection control protocols that included screening for gastrointestinal colonization (11). Unfortunately, suitable media for screening fecal cultures for resistant gram-negative pathogens are not commercially available. Studies examining rates of gastrointestinal carriage of cephalosporin-resistant \textit{Enterobacteriaceae} strains have used locally prepared media, including agar supplemented with ceftaxime (7, 10, 11, 13).

The recent and rapid spread of carbapenem-resistant \textit{Klebsiella pneumoniae}, due to the presence of the carbapenem-hydrolyzing \textit{\beta}-lactamase KPC, in the New York City region has been alarming (1, 2, 4, 16). This pathogen presents a formidable challenge because of its high degree of resistance to virtually all classes of antibiotics (4), and controlling their spread is of utmost importance. In this report, we investigate potential laboratory procedures that could be used by clinical microbiology laboratories for the screening of fecal specimens for these resistant bacteria.

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MATERIALS AND METHODS

Preliminary studies. Six previously characterized clinical strains of carbapenem-resistant \textit{K. pneumoniae}, known to possess \textit{bla}_{KPC-2} were included in experiments to assess the sensitivity of three potential screening methods (1, 4). These six isolates represented the two major ribotypes (two isolates each) involved in outbreaks in New York City, and two isolates from unique ribotypes. Susceptibility testing using Etest, the disk diffusion technique, and the broth microdilution method was performed according to established recommendations (6).

Starting with an initial inoculum of \(\sim 5 \times 10^7 \text{CFU/ml} \) (range, \(1 \times 10^5\) to \(1.7 \times 10^7 \text{CFU/ml}\)), serial 10-fold dilutions of the six isolates were made in normal saline. To include the effect of possible interference from other carbapenem-resistant organisms that can inhabit the gastrointestinal tract, the following organisms were added to each dilution (\(\sim 1 \times 10^8 \text{CFU/ml}\): a clinical isolate of imipenem-resistant \textit{Pseudomonas aeruginosa}, a clinical isolate of vancomycin-resistant \textit{Enterococcus faecium}, and \textit{Candida albicans} ATCC 90028). The culture mixture of each dilution was then processed in three ways. For method 1, 100 \(\mu\)l of the culture mixture was placed in 5 ml of tryptic soy broth containing a 10-\(\mu\)g disk of imipenem. Following an overnight incubation at 37°C, 100 \(\mu\)l was streaked onto MacConkey agar and incubated overnight; the presence or absence of lactose-fermenting gram-negative rods was then recorded. For method 2, 100 \(\mu\)l of the culture mixture was placed in 5 ml of MacConkey broth containing a 10-\(\mu\)g disk of imipenem. Following an overnight incubation at 37°C, 100 \(\mu\)l was streaked onto MacConkey agar and incubated overnight; the presence or absence of lactose-fermenting gram-negative rods was then recorded. For method 3, 100 \(\mu\)l of the culture mixture was placed in 5 ml of tryptic soy broth. Following an overnight incubation at 37°C, 100 \(\mu\)l was streaked onto MacConkey agar and incubated overnight; the presence or absence of lactose-fermenting gram-negative rods was then recorded. For method 2, 100 \(\mu\)l of the culture mixture was placed in 5 ml of tryptic soy broth. Following an overnight incubation at 37°C, 100 \(\mu\)l was streaked onto MacConkey agar and incubated overnight; the presence or absence of lactose-fermenting gram-negative rods was then recorded. For method 3, 100 \(\mu\)l of the culture mixture was placed in 5 ml of tryptic soy broth. Following an overnight incubation at 37°C, 100 \(\mu\)l was streaked onto MacConkey agar and incubated overnight; the presence or absence of lactose-fermenting gram-negative rods was then recorded.

Stool surveillance studies. Surveillance rectal cultures are routinely obtained from all patients in a 10-bed medical-surgical intensive care area as part of an infection control program to identify vancomycin-resistant enterococci. The cultures are performed upon admission to the intensive care area and once weekly during the patients’ stay. Rectal swabs are placed in BBL CultureSwab Plus culturettes (Becton Dickinson, Sparks, MD) containing 5 ml of Amies gel transport medium. During the month of February 2005, an aliquot of transport medium was collected from each culturette before it was discarded. Imipenem-resistant \textit{K. pneumoniae} strains were endemic in the intensive care area during that period. To ensure an equal inoculum for each method, 100 \(\mu\)l of the transport medium was placed into 5 ml of tryptic soy broth and processed according to methods 1 and 3 described above. All gram-negative pathogens in method 1, and all growing within a zone diameter of \(\leq 15\) mm in method 3, underwent imipenem susceptibility testing using the Etest methodology. All imipenem-resistant isolates were then identified using the API 20E system.

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In total, eight samples had imipenem-resistant *K. pneumoniae* (four by both methods and four by method 1 only). In addition, 23 samples had imipenem-resistant *P. aeruginosa*. Of these, 13 were positive by both methods, four were positive by method 1 only, and six were positive by method 3 only.

Fourteen of the 33 patients had more than one surveillance sample taken during the study period. The cultures from these patients were either all positive or all negative in 7 of 14 cases. For the remaining seven patients, the initial culture was negative whereas the subsequent culture(s) grew carbapenem-resistant *K. pneumoniae* or *P. aeruginosa*. The latter patients may have become colonized after the initial culture was taken. No patients with an initially positive culture had a subsequent culture that was negative. Thirteen of the 33 patients had cultures that were all negative. All 13 patients had other (non-surveillance) cultures taken by the medical team as part of their routine care. None of these cultures grew carbapenem-resistant *K. pneumoniae* or *P. aeruginosa*. However, 3 of the 13 patients had routine cultures that grew carbapenem-resistant *A. baumannii* (two from respiratory cultures and one from blood culture).

**DISCUSSION**

Detection of antibiotic-resistant nosocomial pathogens (e.g., vancomycin-resistant enterococci or *Enterobacteriaceae* strains with ESBLs) that commonly colonize the gastrointestinal tract is an integral component of successful infection control protocols (5, 11, 14). Commercially prepared media are available that can be used by clinical laboratories for the detection of vancomycin-resistant enterococci. However, because of the instability of many β-lactams in culture media, no such media exist for the detection of resistant gram-negative pathogens. The difficulty of screening fecal surveillance cultures for bacteria with ESBLs has undoubtedly been an impediment in our efforts to control the spread of these pathogens in the New York City region (15).

Since 2001, our region has also witnessed the rapid spread of carbapenem-resistant *K. pneumoniae*; carbapenem resistance in these isolates is due to the presence of KPC, an efficient class A carbapenem-hydrolyzing β-lactamase (2, 4, 16). Because these pathogens are resistant to virtually all commonly used antibiotics, including β-lactams, fluoroquinolones, aminoglycosides, and not infrequently polymyxins (4), controlling their spread is of utmost importance. As with vancomycin-resistant enterococci and ESBL-possessing *Enterobacteriaceae*, reliance on cultures taken from patients with clinically suspected infection may fail to identify patients harboring KPC-possessing *K. pneumoniae* (2). In outbreak settings, screening for asymptomatic colonization of the gastrointestinal tract will be necessary to identify patients with these pathogens, so that proper infection control efforts can be instituted.

In this report, the medium that consisted of 5 ml of broth containing a 10-μg imipenem disk resulted in the highest recovery of KPC-possessing *K. pneumoniae* from stool surveillance cultures. This method can be easily performed by clinical laboratories. It was apparent that the targeted concentration of imipenem (2 μg/ml, assuming 100% diffusion of the antibiotic) was not achieved with this method, as several of the pathogens isolated by this method had imipenem MICs of between 0.25

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**RESULTS**

**Preliminary studies.** All six clinical isolates used in these studies were intermediate or resistant to imipenem using the recommended inoculum for broth microdilution susceptibility testing (Table 1). However, three isolates had MICs of imipenem that were highly dependent on the inoculum used, a phenomenon frequently observed in KPC-possessing *K. pneumoniae* (4). The lowest limits of detection of six clinical isolates of *K. pneumoniae* with *bla*KPC by the three methods are reported in Table 1. Isolates that had MICs of imipenem that were highly inoculum dependent were more difficult to detect, regardless of the method, whereas isolates that had high MICs of imipenem regardless of inoculum were more easily detected. Methods 1 and 2 yielded comparable results. Method 3 was inferior in detecting three of the isolates (requiring at least 1 log₁₀ CFU/ml more than the other two methods), superior in detecting at least one isolate, and equivalent for the remaining two isolates. Because methods 1 and 2 yielded comparable results, evaluation of the clinical samples was performed by only methods 1 and 3.

**Stool surveillance studies.** Fifty-one stool surveillance cultures from 33 patients underwent testing by methods 1 and 3. For method 1, 13 lactose-fermenting gram-negative bacilli were recovered from 12 samples. Of these 13 isolates, five were imipenem susceptible (MIC range, 0.25 to 3 μg/ml) and eight were resistant to imipenem (MIC, >32 μg/ml). All eight resistant isolates were identified as *K. pneumoniae* with *bla*KPC. In addition, 29 non-lactose-fermenting gram-negative bacilli were recovered. Ten were found to be imipenem susceptible (MIC range, 0.25 to 4 μg/ml). Of the remaining 19 isolates, 17 were *P. aeruginosa* and two were *Acinetobacter baumannii*, all with imipenem MICs of >32 μg/ml.

When the clinical samples were evaluated using method 3, four carbapenem-resistant *K. pneumoniae* strains, all with *bla*KPC, were identified. In addition, 19 imipenem-resistant isolates of *P. aeruginosa* were identified. Since only isolates growing close to the imipenem disk were evaluated, none of the isolates evaluated were found to be imipenem susceptible. *A. baumannii* was not recovered from any of the samples using method 3.

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**TABLE 1. Comparison of three methods for the detection of six clinical isolates of carbapenem-resistant *K. pneumoniae***

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Ribo-type</th>
<th>Imipenem MIC (μg/ml)</th>
<th>Lowest limit of detection (CFU/ml) as determined by in vitro studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC807</td>
<td>A</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>CK850</td>
<td>A</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>OW822</td>
<td>B</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>OW555</td>
<td>B</td>
<td>&gt;32</td>
<td>32</td>
</tr>
<tr>
<td>IC808</td>
<td>C</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>IC855</td>
<td>D</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
</tbody>
</table>

(bioMérieux, France) and screened by PCR for the presence of *bla*KPC using previously defined primers and conditions (1, 4). Amplified *bla*KPC products were identified with bidirectional sequencing, as previously described (1, 4). This study was approved by the Institutional Review Board at SUNY-Downstate Medical Center.
and 4 µg/ml. Confirming imipenem resistance by a disk diffusion assay prior to identification of these isolates would minimize unnecessary work, although it would delay reporting by another 24 h. Increasing the number of imipenem disks placed into the broth may reduce the likelihood of recovering imipenem-susceptible bacteria. The alternative method (plating an overnight growth of the surveillance culture onto MacConkey agar with an imipenem disk and identifying only the isolates near the disk) eliminated the problem of the recovery of carbapenem-susceptible bacteria but appeared less sensitive for the detection of KPC-possessing \textit{K. pneumoniae}. The \textit{K. pneumoniae} has been found in other bacteria, including \textit{Salmonella enterica}, \textit{Klebsiella oxytoca}, and \textit{Enterobacter} spp. (3, 8, 12, 17). An assessment of these methods for detecting other KPC-producing \textit{Enterobacteriaceae} in stool surveillance cultures will have to be performed.

Our region has also been beset with the clonal spread of two other carbapenem-resistant pathogens, \textit{A. baumannii} and \textit{P. aeruginosa} (9). An unexpected finding in our study was the relatively large number of stool samples harboring these isolates, since they are not commonly recognized as inhabitants of the gastrointestinal tract. It appears that the laboratory methods outlined in this study will also be useful in detecting gastrointestinal colonization with these pathogens and assisting infection control efforts.

REFERENCES