Evaluation of CHROMagar KPC for Rapid Detection of Carbapenem-Resistant Enterobacteriaceae

Zmira Samra,1 Judi Bahar,2 Liora Madar-Shapiro,2 Nazi Aziz,1 Sara Israel,2 and Jihad Bishara3*

Laboratory of Clinical Microbiology1 and Unit of Infectious Diseases,3 Rabin Medical Center, Beilinson Hospital, Sackler School of Medicine, Tel-Aviv University, Petah-Tiqwa 49100, Israel, and Hylab, Rehovot, Israel2

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A new CHROMagar KPC medium was compared to MacConkey agar with carbapenem discs and PCR for the blaKPC gene for rapid detection of carbapenem-resistant Klebsiella pneumoniae. The sensitivity and specificity relative to PCR were 100% and 98.4%, respectively, for CHROMagar KPC and 92.7% and 95.9%, respectively, for MacConkey agar.

MacConkey agar were as follows: K. pneumoniae ATCC 13883, Escherichia coli ATCC 8739, Proteus mirabilis ATCC 4630, Salmonella enterica serovar Typhimurium ATCC 14028, Pseudomonas aeruginosa ATCC 27853, and Enterococcus faecalis ATCC 29212. For CHROMagar KPC, three K. pneumoniae extended-spectrum β-lactamase (ESBL)-negative, KPC-positive wild strains and one K. pneumoniae wild strain and one E. coli wild strain, both of which were ESBL positive and KPC negative, were used. All swabs were cultured directly and simultaneously on the reference and CHROMagar KPC plates. The identity of the isolates was confirmed by standard laboratory methods. Subsequently, all swabs were tested for the blaKPC gene by DNA extraction and PCR analysis as described previously (5, 10). Discrepant results between culture and direct PCR were resolved by PCR on colonies. DNA from KPC-positive and KPC-negative strains extracted from swabs was used as a PCR control with each PCR amplification.

Antibiotic susceptibility testing was performed on Mueller-Hinton agar by the disc diffusion method. MICs were defined by the Etest method (AB Biodisk, Solna, Sweden) and tested using isolates obtained directly from CHROMagar KPC before and after subculture on MacConkey agar. MIC breakpoints were defined according to Clinical and Laboratory Standards Institute (CLSI) criteria (3). ESBL production was tested using the cefotaxime-ceftazidime–clavulanic acid double-disc method as previously described (1).

Of the 122 swabs, 79 were negative by both culture and PCR. Forty-three typical K. pneumoniae mucoid metallic blue colonies grew on CHROMagar KPC. Among these, two were ESBL positive, resistant only to ertapenem, and lacked the blaKPC gene. The MacConkey agar test failed to detect CR K. pneumoniae in three swabs. Although CR K. pneumoniae concentrations in these samples were low, they were detected with CHROMagar KPC. Direct PCR failed to detect the KPC gene in another six CR K. pneumoniae swabs. Few colonies grew when these swabs were first cultured. However, corresponding colonies were found to be KPC positive by PCR (Table 1). Since our swabs were first used for culturing on MacConkey agar and CHROMagar KPC and subsequently for PCR, we assume that residue from swabs with low inocula was not sufficient for successful DNA extraction, leading to false-neg-

*Corresponding author. Mailing address: Infectious Diseases Unit, Rabin Medical Center, Beilinson Hospital, Petah-Tiqwa 49100, Israel. Phone: (972) 3-937-7511. Fax: (972) 3-937-7513. E-mail: bishara@netvision.net.il.

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TABLE 1. Detection of CR *Klebsiella pneumoniae* isolates from stool specimens by three methods

<table>
<thead>
<tr>
<th>No. of specimens</th>
<th>MacConkey agar + carbapenem disc</th>
<th>CHROMagar KPC</th>
<th>PCR swab</th>
<th>PCR colony</th>
</tr>
</thead>
<tbody>
<tr>
<td>79</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Not tested</td>
</tr>
<tr>
<td>32</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Not tested</td>
</tr>
<tr>
<td>6</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>Not tested</td>
</tr>
<tr>
<td>2</td>
<td>Ertapenem resistant(^b)</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

\(^a\) A total of 122 specimens were tested; 43 colonies grew on CHROMagar KPC; and 35 swabs were tested by PCR.

\(^b\) Ertapenem resistant, ertapenem-resistant isolates detected.

ative KPC results by PCR. Hence any discrepancies between culture and PCR were resolved by KPC PCR on the corresponding colonies. An internal control to assess inhibition of PCR was not included in every sample.

The sensitivity and specificity for detecting CR *K. pneumoniae*, relative to PCR, were 100% and 98.4%, respectively, for CHROMagar KPC and 92.7% and 95.9%, respectively, for MacConkey agar.

The 41 *K. pneumoniae* ESBL-negative and KPC-3-positive isolates were found to be resistant to all classes of antibiotics, except gentamicin and colistin. MICs of all carbapenems were \(\geq 16\) µg/ml. Only one isolate was resistant to tigecycline, with a MIC of 16 µg/ml. The two ESBL-positive isolates resistant only to ertapenem were sensitive to imipenem, meropenem, gentamicin, and colistin but resistant to tigecycline, with a MIC of 16 µg/ml, and all other tested antibiotics. It is noteworthy that identical MICs for tested isolates obtained directly from CHROMagar KPC before and after subculture were observed.

This is the first study describing the use of a new CHROMagar KPC medium for rapid and direct detection of CR *K. pneumoniae* from clinical specimens. CHROMagar KPC demonstrated an excellent ability to detect CR *K. pneumoniae*. The color and morphology characteristics on CHROMagar KPC permit easy differentiation of the bacterial colonies. Moreover, this medium allows detection of isolates resistant to ertapenem and sensitive to other carbapenems without the need for subculturing.

Previous studies reporting gastrointestinal tract carriage of cephalosporin-resistant *Enterobacteriaceae* spp. used ceftaxime-containing agar (6–8). Landman et al. (4) investigated a medium containing imipenem discs for detecting CR *K. pneumoniae* strains, finding that a method using broth containing an imipenem disk had greater sensitivity for detecting CR *K. pneumoniae* than two other methods of plating a surveillance culture onto the MacConkey agar. In another study, Bratu et al. (2) applied the PCR method to detect KPC-2-positive *E. coli* stool isolates. In these studies, the CHROMagar KPC medium obviously was not evaluated.

Our study has two major limitations: the sample size was small, and the study was performed at one institution and involved predominantly a single clone of CR *K. pneumoniae*. Our results should be interpreted with caution; use of multiple clones with various carbapenem MICs might affect the results.

Further studies will be needed to establish the reliability of the new CHROMagar KPC medium and its applicability for detecting other CR gram-negative pathogens, such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Since the *bla*KPC gene plays a pivotal role in the spread of CRE infection, its presence should be confirmed only by PCR-based methods, either from colonies grown on CHROMagar KPC or directly from fresh swabs. An internal control should be included in the PCR for every sample to preclude false-negative results due to inhibition of PCR.

REFERENCES


