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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 Bishara[3]*

Laboratory of Clinical Microbiology[1] 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, Israel[2]

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A new CHROMagar KPC medium was compared to MacConkey agar with carbapenem discs and PCR for the *bla*$_{KPC}$ 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.

Rapid laboratory detection of carbapenem-resistant *Enterobacteriaceae* (CRE) in hospitalized patients is highly desirable in areas where these strains are endemic. Culturing on MacConkey agar supplemented with carbapenems is easy to perform and is a cheap method of CRE detection, but is time-consuming. PCR, although a highly sensitive and specific method, is unavailable for daily use in many laboratories (2, 4). A new chromogenic medium, CHROMagar KPC, commercially unavailable, has been recently developed. It is CHROMagar Orientation (7, 9) supplemented with agents that inhibit the growth of gram-positive/gram-negative carbapenem-sensitive bacteria. After incubation of 24 h at 37°C, CRE cultures took on different colors according to their specific enzymatic properties.

Our aim was to evaluate the sensitivity and specificity of this medium in identifying carbapenem-resistant (CR) *Klebsiella pneumoniae* compared to MacConkey agar with carbapenem discs and PCR for *bla*$_{KPC}$ genes. One hundred twenty-two patient-unique rectal swabs were tested.

In our institution, infection control personnel uninvolved in the study chose rectal swabs as the most appropriate source of isolates for the study. Investigators who performed PCR assays were blinded as to the results of the CHROMagar KPC and MacConkey agar cultures and vice versa.

Dehydrated powder of the CHROMagar KPC was provided by the CHROMagar Company, Paris, France. The medium was prepared by Hy-Laboratories, Rehovot, Israel, according to the manufacturer's instructions and poured into 90-mm-diameter petri dishes, stored at 4 to 6°C, protected from light, and used within 10 weeks. Standard reference medium consisted of MacConkey agar (Difco) prepared according to the manufacturer's instructions and used with imipenem (10 μg), meropenem (10 μg), and ertapenem (10 μg) discs (Oxoid). Carbapenem discs were placed on culture medium at the 4-, 8-, and 12-o'clock positions. Each batch of culture medium was checked for growth promotion from minimum inocula (10 to 100 CFU) and inhibitory and indicative properties according to specific standard operation procedures. ATCC strains for 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 *bla*$_{KPC}$ 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 *bla*$_{KPC}$ 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[a]

No. of specimens	Result by test with:			
	MacConkey agar + carbapenem disc	CHROMagar KPC	PCR swab	PCR colony
79	Negative	Negative	Negative	Not tested
32	Positive	Positive	Positive	Not tested
6	Positive	Positive	Negative	Positive
3	Negative	Positive	Positive	Not tested
2	Ertapenem resistant[b]	Positive	Negative	Negative

[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 ≥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 cefotaxime-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.

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