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Evaluation of CHROMagar™ KPC for the detection of carbapenemase-producing Enterobacteriaceae in rectal surveillance cultures

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ABSTRACT

In this study, the performance of the chromogenic medium CHROMagar™ KPC was evaluated and was compared with in-house-daily prepared McConkey agar plates supplemented with imipenem (1 mg/L) for the detection of carbapenemase-producing Enterobacteriaceae. In this surveillance study, rectal swabs were cultured on both media and polymerase chain reaction (PCR) for bla_{KPC} and bla_{VIM} was used to confirm the genotype of growing colonies of Enterobacteriaceae. CHROMagar KPC was also tested with 17 genotypically characterised carbapenemase-producing and non-producing Gram-negative bacteria. It was shown that CHROMagar allows rapid detection of carbapenemase-producing Enterobacteriaceae, although bla_{KPC}- and bla_{VIM}-harbouring isolates could not be differentiated by colour or colony morphology. The positive and negative predictive values of the tested methods for the detection of carbapenemase-producing Enterobacteriaceae were, respectively, 100% and 98.8% for CHROMagar KPC and 94.7% and 88.6% for imipenem-supplemented McConkey agar. CHROMagar KPC medium is a useful screening medium for carbapenemase-producing Enterobacteriaceae in stools in settings with a high proportion of patients colonised with a variety of carbapenemase-producers.

1. Introduction

Infections caused by carbapenem-resistant Enterobacteriaceae are an emerging problem associated with high rates of morbidity and mortality, particularly amongst critically ill patients [1,2]. Carbapenem-resistant Enterobacteriaceae are usually resistant not only to β-lactam antimicrobials but also to most other classes of antimicrobial agents [2].

The most important mechanism of resistance to carbapenems is carbapenemase production. KPC and VIM are the most prevalent carbapenemases produced by Enterobacteriaceae not only in Greece but also in several other countries worldwide [3–8].

Patients colonised with carbapenem-resistant Enterobacteriaceae are thought to be the source of transmission in healthcare settings [7,9]. Surveillance cultures are useful in identifying such patients in order to implement infection control measures rapidly. Accurate detection of colonisation with carbapenem-resistant Enterobacteriaceae within a short time from sampling contributes to the effectiveness of the above measures as well as to the adequacy of the prescribed empirical antimicrobial treatment in severely ill patients [10,11].

Commercially prepared media for isolation of vancomycin-resistant enterococci and meticillin-resistant *Staphylococcus aureus* (MRSA) have facilitated cultivation techniques for rapid evaluation of gastrointestinal colonisation. Until recently, detection of carbapenem-resistant Enterobacteriaceae has been troublesome and was performed using in-house-prepared selective media such as agar or tryptic soy broth containing a 10 μg disk of imipenem, meropenem or ertapenem [9,11,12] or with polymerase chain reaction (PCR)-based techniques [13], each with its own advantages and disadvantages. CHROMagar™ KPC (Hy-Labs, Rehovot, Israel) is a commercially prepared chromogenic solid medium supplemented with agents that inhibit the growth of carbapenem-sensitive bacteria. Following 24 h of incubation, carbapenem-resistant Enterobacteriaceae colonies appear with different colours according to their specific enzymatic properties: *Escherichia coli* appear as red colonies, *Klebsiella* spp., *Enterobacter* spp. and *Citrobacter* spp. as metallic blue and *Pseudomonas* spp. as translucent cream colonies [14].

In the Intensive Care Unit (ICU) of University General Hospital 'Attikon' (Athens, Greece), in order to achieve early detection of carbapenem-resistant Gram-negative bacteria, surveillance

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Table 1
Colony morphology of genotypically characterised carbapenem-resistant strains on CHROMagar™ KPC.

Microorganism	Genotype[a]	MIC (mg/L)			Colony morphology
		IPM	MEM	ERT	
Escherichia coli	bla_{VIM}	8	1	1	Poor growth, small pink colonies
E. coli	bla_{KPC}	>8	>8	>4	Pink colonies
Klebsiella pneumoniae	bla_{KPC}	>8	>8	>4	Steel blue colonies
K. pneumoniae	bla_{KPC}	>8	>8	>4	Steel blue colonies
K. pneumoniae	bla_{VIM}	>8	8	>4	Steel blue colonies
Enterobacter cloacae	bla_{VIM}	2	>8	>4	Steel blue colonies
E. cloacae	bla_{VIM} and bla_{KPC}	>8	8	4	Steel blue colonies
Enterobacter aerogenes	bla_{KPC}	>8	>8	>4	Steel blue colonies
E. aerogenes	bla_{KPC}	>8	>8	>4	Steel blue colonies
Citrobacter freundii	bla_{VIM}	4	≤1	2	Poor growth, small pink colonies with dark centre
Proteus mirabilis	bla_{VIM}	>8	>8	>4	Colonies with brown halo
P. mirabilis	bla_{VIM}	4	4	0.5	Colonies with brown halo
Pseudomonas aeruginosa	bla_{VIM}	>8	>8	N/D	White colonies
P. aeruginosa	None	4	>8	N/D	White colonies
P. aeruginosa	None	4	>8	N/D	White colonies
Acinetobacter baumannii	bla_{VIM}	>8	>8	>8	White colonies
A. baumannii	bla_{OXA-58}	>8	>8	>8	White colonies

MIC, minimum inhibitory concentration; IPM, imipenem; MEM, meropenem; ERT, ertapenem; N/D, not determined.
[a] Genotype was confirmed by polymerase chain reaction (PCR) using specific primers and sequencing [15–17].

cultures (rectal swabs and bronchial secretions) are performed twice weekly in all ICU patients. Samples are plated on in-house-prepared McConkey agar supplemented with antibiotics. In the present study, the performance of CHROMagar KPC was compared with that of the in-house-daily prepared McConkey agar plates supplemented with imipenem for the detection of carbapenem-resistant Enterobacteriaceae.

2. Material and methods

2.1. Preliminary studies

To evaluate the reliability of the new CHROMagar KPC and its applicability for detecting carbapenem-resistant Gram-negative pathogens harbouring different resistance mechanisms, 17 genotypically characterised clinical isolates were studied, including 12 carbapenemase-producing Enterobacteriaceae, 3 carbapenemase-producing non-fermenters and 2 meropenem-resistant non-carbapenemase producing *Pseudomonas aeruginosa* isolates (Table 1). The colour and morphological characteristics of the colonies grown on CHROMagar KPC were recorded after 24 h and 48 h of incubation in ambient air at 35 °C.

2.2. Comparative evaluation of screening methods

Rectal swabs from patients hospitalised in four different tertiary hospitals in Athens, Greece, during February 2009 were promptly placed in Stuart transport medium (Cultiplast; LPT Italiana SpA, Milan, Italy) and were transported within 12 h to the Clinical Microbiology and Infectious Diseases Laboratory of the 4th Department of Internal Medicine, University General Hospital 'Attikon'. Swabs were then plated sequentially both on commercially prepared CHROMagar KPC and on in-house-daily prepared McConkey agar plates (Becton-Dickinson, Cockeysville, MD) supplemented with 1 mg/L imipenem. To ensure equal inoculation, each swab was plated alternately on both media. Plates were incubated at 35 °C in ambient air and were examined for growth at 24 h and 48 h. All morphologically different colonies from imipenem-supplemented McConkey agar and all differently coloured colonies (blue, red or with a brown halo according to the manufacturer) from CHROMagar KPC were subcultured on McConkey agar and were submitted to identification and susceptibility testing using a BD Phoenix automated microbiology system (Becton Dickinson Diagnostic Systems, Sparks, MD). Metallo-β-lactamase and KPC production were screened using the ethylene diamine tetra-acetic acid (EDTA)–imipenem approximation disk synergy test and the boronic acid disk test, respectively [18,19]. The presence of bla_{KPC} and bla_{VIM} was confirmed by PCR with specific primers [15,16].

2.3. Detection limit

Four genotypically characterised clinical strains of carbapenem-resistant *Klebsiella pneumoniae* [imipenem minimum inhibitory concentration (MIC) ≥16 mg/L] known to possess bla_{KPC-2} or bla_{VIM-1} were included in the experiments to assess the detection limit of the CHROMagar KPC screening method. Starting with an initial inoculum of ca. 5×10^7 colony-forming units (CFU)/mL, serial 10-fold dilutions of the four *K. pneumoniae* isolates were prepared in normal saline. Viable cells were counted by the colony count technique according to Clinical and Laboratory Standards Institute (CLSI) methodology [20].

To assess possible interference from other organisms that can inhabit the gastrointestinal tract, the following organisms were added to each dilution (ca. 5×10^7 CFU/mL): a clinical isolate of imipenem-resistant *P. aeruginosa* harbouring bla_{VIM-1}; a clinical isolate of extended-spectrum β-lactamase-producing *K. pneumoniae*; and *E. coli* ATCC 35218. Then, 100 μL of each of the culture mixtures was plated on CHROMagar KPC and incubated for 24–48 h for colony counts; recovery of steel blue colonies was recorded. The detection limit of CHROMagar KPC was the lowest concentration of the isolate that resulted in recovery of steel blue colonies.

2.4. Analysis of the results

Swabs positive for any carbapenemase-producing Enterobacteriaceae by both methods (red or metallic blue colonies on CHROMagar KPC and growth on imipenem-supplemented McConkey agar and a positive PCR for bla_{VIM} and/or bla_{KPC}) were characterised as 'true positive' and those negative by both methods (no red or metallic blue colonies on CHROMagar KPC and no growth of Enterobacteriaceae on imipenem-supplemented McConkey agar) were characterised as 'true negative'. All swabs yielding discrepant results between the two testing methods were finally characterised as 'true positive' or 'true negative' with regard to the presence of carbapenemase-producing Enterobacteriaceae by PCR for bla_{VIM} and bla_{KPC}, as described above (Fig. 1). The posi-

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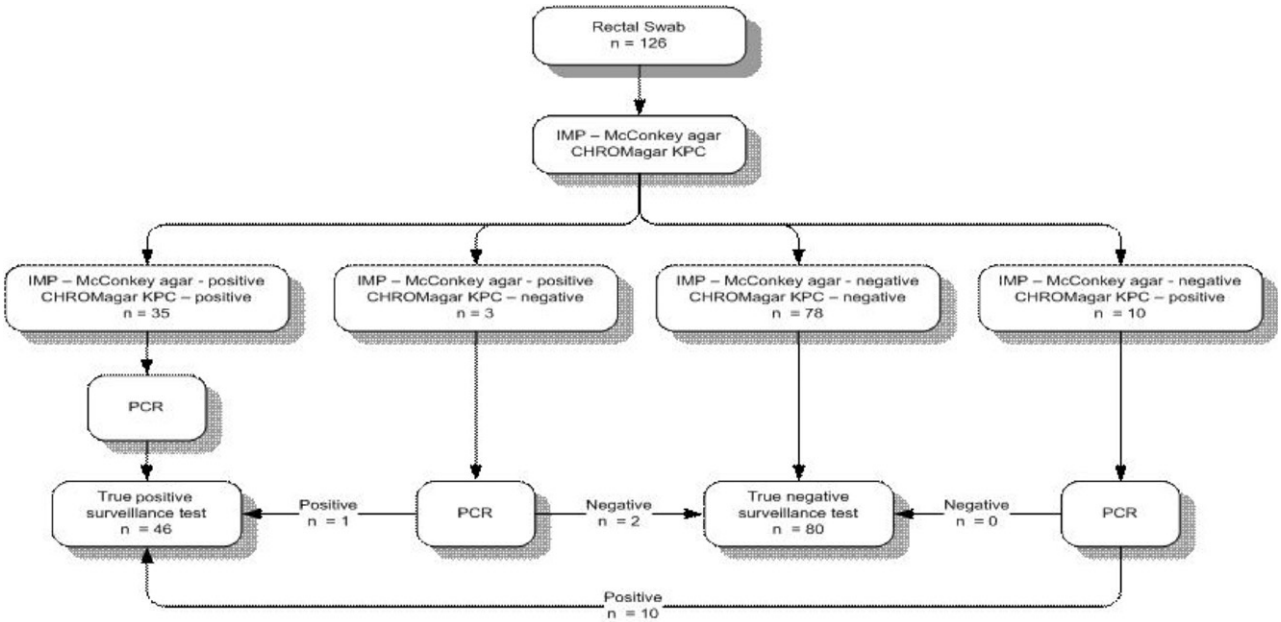

Fig. 1. Processing of 126 rectal swabs by culture on imipenem-containing McConkey agar (IMP-McConkey agar) and CHROMagar™ KPC for the detection of carbapenemase-producing Enterobacteriaceae, and final interpretation of the results by polymerase chain reaction (PCR) using bla_{KPC}- and bla_{VIM}-specific primers.

tive predictive value (PPV) and negative predictive value (NPV) of both methods was calculated.

3. Results

3.1. Preliminary studies

All tested strains grew on CHROMagar KPC. *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Enterobacter aerogenes* harbouring bla_{VIM} and/or bla_{KPC} yielded large steel blue colonies at 24 h. bla_{VIM}- or bla_{KPC}-harbouring *E. coli* and *Citrobacter freundii* grew as pink colonies or pink with darker centre colonies, respectively, whereas *Proteus mirabilis* grew as colonies with a brown halo. The two types of carbapenemases (VIM or KPC) could not be differentiated by the colony morphology of producing strains (Table 1). The single strain of *E. cloacae* harbouring both carbapenemases was recently isolated from rectal surveillance specimens of two ICU patients (unpublished data).

3.2. Comparative evaluation of screening methods

In total, 126 rectal swabs from 121 patients hospitalised in four different tertiary hospitals in Athens during February 2009 were examined. A second sample collected during a 15-day period from five patients was included in the study because of changes in the recovered microbial flora. Of the 121 patients, 79 (65.3%) were in the ICU and 42 (34.7%) were in internal medicine and surgical wards.

3.2.1. Imipenem-supplemented McConkey agar

Amongst 126 samples cultured, 38 (30.2%) yielded at least one strain of the family Enterobacteriaceae, 66 samples (52.4%) yielded no growth (Fig. 1) and the remaining 22 samples (17.5%) grew only non-Enterobacteriaceae. In total, 93 isolates were recovered from imipenem-supplemented McConkey agar. Of 44 Enterobacteriaceae strains, 4 were imipenem- and meropenem-susceptible [21] and were identified as *E. coli* negative for bla_{VIM} or bla_{KPC} by PCR (2 of them were present in the sample with carbapenemase-producing Enterobacteriaceae). The other 40 isolates were non-susceptible to imipenem and/or meropenem and were identified as KPC-producing *K. pneumoniae* ($n = 30$), VIM-producing *K. pneumoniae* ($n = 7$), KPC-producing *Enterobacter* spp. ($n = 2$; both coexisted in the sample with KPC-producing *K. pneumoniae*) and VIM-producing *P. mirabilis* ($n = 1$). Amongst 49 non-fermenting Gram-negative bacilli, 9 were found to be imipenem-susceptible and were identified as *P. aeruginosa*; the remaining 40 carbapenem-non-susceptible isolates were identified as *P. aeruginosa* or *Acinetobacter baumannii*.

3.2.2. CHROMagar KPC

Metallic blue colonies were obtained from 45 samples (Fig. 1). In 10 rectal samples, CHROMagar KPC detected carbapenem-resistant *K. pneumoniae* harbouring bla_{KPC} ($n = 6$) or bla_{VIM} ($n = 4$) that the in-house-prepared imipenem-supplemented McConkey agar plate failed to detect (Fig. 1). In total, 47 isolates were recovered, all identified as *K. pneumoniae*. Forty-six were imipenem- and/or meropenem-resistant and one was imipenem-intermediate (MIC = 8 mg/L) and meropenem-susceptible (MIC = 2 mg/L). Thirty-five isolates (74.5%) harboured bla_{KPC} and 12 isolates (25.5%) harboured bla_{VIM}. White and light green colonies were also recovered from 35 samples but were not tested further as they were thought to be non-Enterobacteriaceae.

Concordant results between the two methods were documented for 113 specimens (89.7%), whereas discordant results were recorded for 13 specimens (10.3%). The PPV and NPV of the tested methods for the detection of carbapenemase-producing Enterobacteriaceae were, respectively, 100% and 98.8% for CHROMagar KPC and 94.7% and 88.6% for imipenem-supplemented McConkey agar (Fig. 1).

Moreover, 42 (91.3%) of the 46 true positive samples were recorded in ICU patients.

All carbapenem-non-susceptible *K. pneumoniae* strains were isolated after 24 h of incubation on either agar plate, except for two isolates that grew the first day on CHROMagar KPC and the second day on imipenem-supplemented McConkey agar.

Klebsiella pneumoniae isolates harbouring bla_{KPC} were 100% non-susceptible to imipenem and 94.6% non-susceptible to meropenem, whilst those harbouring bla_{VIM} were 92.9% non-susceptible to

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imipenem and 50% non-susceptible to meropenem according to CLSI breakpoints [21] (Table 2).

3.3. Detection limit experiment

The two KPC-2-producing *K. pneumoniae* isolates were detected as metallic blue colonies on CHROMagar KPC with a detection limit of 4×10^1–9×10^2 CFU/mL, whilst for the two VIM-1-producing *K. pneumoniae* strains the detection limit was 7×10^2–5×10^3 CFU/mL.

4. Discussion

Rapid detection of antibiotic-resistant nosocomial pathogens in the gastrointestinal tracts of patients is considered a necessary step in successful infection control protocols [2,22]. It provides useful information on the prevalence of colonisation by multiresistant pathogens and dictates strict implementation of additional infection control measures. Obtaining results in a short time interval and taking immediate action in terms of infection control prevents further dissemination of resistant strains as well as predicting the real pathogen in the case of ventilator-associated pneumonia or bacteraemia [10].

In the present study, CHROMagar KPC medium was evaluated for its performance in detecting 17 genotypically characterised clinical isolates. It was shown that all tested Gram-negative bacteria grew on CHROMagar KPC and it was possible to detect KPC and/or VIM carbapenemase-producing Enterobacteriaceae in just 24 h. Nevertheless, differentiation between *bla*$_{KPC}$- and *bla*$_{VIM}$-harbouring Enterobacteriaceae was not possible by colour or colony morphology. Likewise, differentiation of carbapenemase-producing *P. aeruginosa* from meropenem-non-susceptible strains not producing a carbapenemase was not possible. *Citrobacter freundii* and *E. coli* strains with low MICs to carbapenems (intermediate or susceptible) showed poor growth even after 48 h of incubation and were less efficiently detected (Table 1).

In this surveillance study conducted in four institutions with a high percentage of patients colonised with *bla*$_{KPC}$- and/or *bla*$_{VIM}$-positive Enterobacteriaceae (true positive specimens, 36.5%), CHROMagar KPC showed an excellent PPV and NPV of 100% and 98.8%, respectively, in detecting carbapenemase-producing Enterobacteriaceae compared with in-house-prepared medium that showed a PPV of 94.7% and a NPV of 88.6%. The lower NPV of McConkey agar necessitates confirmatory PCR testing and final results are available ≥48 h after sampling.

It should be noted that with the use of imipenem-supplemented McConkey agar it was possible to distinguish a variety of carbapenemase-producing species of Enterobacteriaceae growing simultaneously on the plate owing to the different colony morphologies, whereas using CHROMagar KPC carbapenemase-producing *K. pneumoniae* and carbapenemase-producing *Enterobacter* spp. present in the same sample were indistinguishable. *Klebsiella pneumoniae bla*$_{KPC}$- or *bla*$_{VIM}$-harbouring isolates could not be differentiated by colour or colony morphology in either of the tested media. For these reasons, CHROMagar KPC did not detect 2 of the 37 KPC-producing and 2 of the 14 VIM-producing *K. pneumoniae* strains that were isolated from imipenem-supplemented McConkey agar because they coexisted with other carbapenemase-producers. CHROMagar failed to detect one *bla*$_{VIM}$-positive *P. mirabilis* isolate that was recovered on imipenem-supplemented McConkey agar with imipenem and meropenem MICs of ≥16 mg/L since *P. mirabilis* colonies producing a brown halo could be missed in a heavy growth of blue and white colonies.

In this study, the detection limit was determined only for CHROMagar KPC, which was able to detect low concentrations of

Table 2
Recovery and minimum inhibitory concentration (MIC) distribution of *Klebsiella pneumoniae* strains isolated using imipenem-supplemented McConkey agar and CHROMagar™ KPC.

Isolates	Recovered on:	No. of strains	MIC (mg/L)									
			Imipenem (no. of strains)					Meropenem (no. of strains)				
			≤1	2	4	8	≥16	≤1	2	4	8	≥16
KPC-producing *K. pneumoniae* (*N* = 37)	Both plates	28	–	–	–	4	24	–	–	–	–	28
	Imipenem-supplemented McConkey agar only	2	–	–	–	–	2	–	–	–	–	2
	CHROMagar KPC only	7	–	–	–	2	5	–	2	–	–	5
VIM-producing *K. pneumoniae* (*N* = 14)	Both plates	5	–	–	1	1	3	–	–	1	1	3
	Imipenem-supplemented McConkey agar only	2	–	–	–	–	2	–	–	–	–	2
	CHROMagar KPC only	7	–	–	–	–	7	4	1	1	1	–

KPC-2- and VIM-1-producing *K. pneumoniae* in stools (4×10^1 and 7×10^2 CFU/mL, respectively).

Until now, detection of carbapenem-resistant Enterobacteriaceae in stools has been mainly performed with in-house-prepared media and the use of carbapenems either in disks on the surface of the agar or diluted in the agar or with PCR-based techniques. The instability of β-lactam antimicrobials added to the medium is a problem that needs to be overcome. For this purpose, daily fresh-prepared imipenem-supplemented McConkey agar plates were used in University General Hospital 'Attikon'. By this method, confirmed results were available in 48 h. The use of disks on the surface of the agar in institutions where carbapenem-resistant microorganisms are endemic causes further delay, since crowding of colonies close to the carbapenem disk necessitates subcultures in order to isolate and test growing colonies.

The US Centers for Disease Control and Prevention (CDC) recently proposed a protocol for the detection of carbapenem-resistant *Klebsiella* spp. and *E. coli* in stool, according to which samples are first inoculated in trypticase soy broth with a meropenem or ertapenem disk, incubated overnight and subsequently cultured on McConkey agar. Lactose-fermenting colonies are then screened for carbapenemase production using a phenotypic test such as the modified Hodge test [11]. This method has been shown to detect carbapenem-resistant *K. pneumoniae* KPC-2 in concentrations ranging between 1.7×10^6 to 2.7×10^0, depending on the imipenem MIC of the strain [12]. Results by this method are available after not less than 3–4 days, therefore delaying the implementation of appropriate infection control measures.

Samra et al. [23] compared CHROMagar KPC to McConkey agar with carbapenem disks and direct PCR for bla_{KPC} for rapid detection of carbapenem-resistant KPC-producing Enterobacteriaceae from rectal swabs. The sensitivity and specificity relative to PCR were 100% and 98.4%, respectively, for CHROMagar KPC and 92.7% and 95.9%, respectively, for McConkey agar. Imipenem-supplemented McConkey agar was evaluated by Schechner et al. [13] for the detection of KPC-producing Enterobacteriaceae from rectal swabs in comparison with PCR for bla_{KPC}. The sensitivity and specificity of that medium were 87.5% and 99.4%, respectively. PCR showed higher sensitivity and specificity and provided confirmed results sooner [13]. Nevertheless, PCR is an expensive method to use routinely and requires qualified personnel. In Greece, commercially available PCR kits detecting KPC and VIM carbapenemases cost ca. €40 per sample, whilst CHROMagar and imipenem-supplemented McConkey agar plates cost €8 and €0.40, respectively.

To our knowledge, this is the first study evaluating the performance of CHROMagar KPC medium in the detection of VIM-producing Enterobacteriaceae in rectal samples. A potential limitation of this study is the absence of any reference criterion by which samples may be characterised as truly negative. Therefore, the true NPV may be lower than reported. The reliability and applicability for the detection of other carbapenem-resistant Gram-negative bacilli, such as *Acinetobacter* spp. and *P. aeruginosa*, need to be evaluated for both of the studied media.

In conclusion, CHROMagar KPC medium is a very useful screening medium both for KPC and VIM carbapenemase-producing Enterobacteriaceae in stools. Taking into account that the time to detection of resistance is crucial in infection control policies, CHROMagar KPC, being much cheaper than PCR, efficiently identifies colonised patients in a much shorter time compared with imipenem-supplemented McConkey agar, thus permitting immediate implementation of infection control measures to prevent further dissemination, and in the case of infection directs therapy away from β-lactam antibiotics.

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