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Comparison of Two Chromogenic Media and MacConkey Agar with Carbapenem Disks for the Detection of Carpabenemase-Producing Gram Negative Rods in Simulated Stool Specimens

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Background

Carbapenemase producing Gram negative bacilli (CPGNR) represent a major threat in healthcare settings as infections caused by these bacteria are associated with higher mortality rates in part because of extremely limited treatment options[1]. Screening of stool specimens is recommended by the Center for Disease Control (CDC) to identify carriers and initiate appropriate infection control measures to prevent their spread[2]. Therefore, clinical laboratories must be able to rapidly and accurately screen stools and rectal swabs for the presence of CPGNR.

Laboratory techniques and algorithms have been published for the detection of CPGNR from rectal swabs[3-4-5]. The CDC has proposed a two-step method based on broth enrichment in a medium containing either 10-µg imipenem or meropenem disks followed by selective subculture on MacConkey agar[6]. This method is easy to perform but has a long turn-around time (96 hours). Molecular testing using in house PCR protocols has proven rapid and accurate but this technology is not widely available in clinical laboratories[7-8]. More recently, selective and differential chromogenic agars, such as CHROMagar KPC and ChromID ESBL, have been proposed as rapid and simple culture media for the screening of clinical specimens[9-10-11-12]. Direct plating of specimens onto MacConkey agar with carbapenem disks has also proven effective. Lolans and colleagues reported that an ertapenem zone diameter of ≤27mm was highly sensitive for detection of KPC-producing Enterobacteriaceae in rectal swab specimens[13]. However, the zone diameter interpretive criteria for imipenem and meropenem directly put on MacConkey agar have not been established yet.

The objectives of this study were twofold: i) to compare the performance of CHROMagar KPC, ChromID ESBL and MacConkey agar + 10-µg carbapenem disks (ertapenem, meropenem and imipenem) for the detection of CPGNR in simulated stool specimens and ii) to define the optimal carbapenem inhibition zone diameters for detecting CPGNR when using disks directly placed on MacConkey agar.

Methods

Bacterial isolates

39 clinical isolates were obtained from the Laboratoire de Santé Publique du Québec (LSPQ) microbial collection. These strains have been well characterized, phenotypically and genotypically (LSPQ, National Microbiology Laboratory, Public Health Agency of Canada). 20 CPGNR isolates (17 *Enterobacteriaceae* and 3 Non-Fermenters) were selected upon the presence of genes coding for different carbapenemases, namely: KPC, NDM, OXA, VIM, IMP and NMC. 19 non-CPGNR were also selected as negative control (18 ESBL or AmpC producing *Enterobacteriaceae* as well as a susceptible wild-type *Escherichia coli* strain). CPGNR with a wide range of minimal inhibitory concentrations (MICs) to ertapenem, meropenem and imipenem were selected in order to better assess the performance of the different screening methods. The MICs of ceftazidime, cefotaxime, ertapenem, meropenem and imipenem were determined by the microdilution method according to the Clinical and Laboratory Standards Institute (CLSI).

Selective media

Five screening agar plates were tested: ChromID ESBL agar plate containing a chromogenic substrate and cefpodoxime (BioMerieux, Marcy l'Étoile, France), CHROMAgar KPC agar plate containing a chromogenic substrate and a carbapenem antibiotic (Alere Inc, Canada) and three MacConkey agar plates with either ertapenem (10 µg), or meropenem (10 µg) or imipenem (10 µg) disks. The carbapenem disks were purchased from MAST Diagnostics (MAST Group Ltd, Merseyside, UK) and the chromogenic agar plates provided free of charge by the manufacturers.

Fecal material

A single stool specimen obtained from a normal volunteer was used to prepare all the simulated clinical specimens. Briefly, the stool was liquefied in 0,85% normal saline and aliquots of 900 µl were prepared and stored at – 20° C. To ensure that the specimen did not harbour any beta-lactam resistant bacteria, screening tests were performed using ChromID ESBL, CHROMagar KPC and MacConkey agar with ertapenem, meropenem and imipenem disks. Each plate was inoculated with 100 µl of liquefied stool and incubated 24 hours in ambient air at 35° C. There was no growth on the two selective chromogenic agar plates (ChromID ESBL and CHROMagar KPC). For the Mac Conkey agar, inhibition diameters around the antibiotic disks were 29 mm, 39 mm and 35 mm respectively for ertapenem, meropenem, and imipenem.

Screening of carbapenemase resistant isolates

In order to determine the sensitivity and specificity of the screening assays, serial 10-fold dilutions of the 39 isolates were prepared in 0,85% normal saline and 100 µl of each dilution was inoculated into 900 µl aliquots of stool. The simulated infected fecal material was inoculated onto the five screening media to obtain a final challenge concentrations of 10^4 to 10^1 CFU/ml for each strain. The fecal inoculum was deposed in the central zone of the agar plates and spread by rotation as evenly as possible using a rake spreader. Antibiotic disks of each of the three carbapenems were individually place onto MacConkey agar. All media were incubated aerobically at 35° C for 24 hours. The plates were examined for growth and, if pre-sent, the colour and morphological characteristics of the colonies that grew on ChromID ESBL and CHROMAgar KPC were recorded and the diameter of the inhibition zones around each carbapenem disk on MacConkey agar plates measured.

Results

TABLE 1
Characteristics of the bacterial isolates

Isolate	Type of β-lactamase	Gene	MIC (ug/ml)				
			CAZ	CTX	ERTA	MERO	IMI
Citrobacter freundii	Carbapenemase	KPC	> 64	> 32	16	16	16
Enterobacter cloacae	Carbapenemase	NMC	0.5	1	16	16	32
Enterobacter cloacae	Carbapenemase	KPC	> 64	> 32	8	4	4
Escherichia coli	Carbapenemase	KPC	> 64	> 32	8	4	16
Escherichia coli	Carbapenemase	KPC	> 64	> 32	4	4	4
Escherichia coli	Carbapenemase	KPC	> 64	> 32	4	4	8
Escherichia coli	Carbapenemase	KPC	> 64	> 32	4	4	16
Klebsiella pneumoniae	Carbapenemase	KPC	> 64	> 32	> 32	> 32	> 32
Klebsiella pneumoniae	Carbapenemase	KPC	> 64	> 32	> 32	> 32	> 32
Klebsiella pneumoniae	Carbapenemase	KPC	> 64	> 32	> 32	> 32	> 32
Klebsiella pneumoniae	Carbapenemase	KPC	> 64	> 32	> 32	> 32	> 32
Klebsiella pneumoniae	Carbapenemase	KPC	> 64	> 32	> 32	> 32	> 32
Klebsiella pneumoniae	Carbapenemase	OXA-48	1	1	4	2	8
Klebsiella pneumoniae	Carbapenemase	NDM-1	> 64	> 32	> 32	> 32	> 32
Klebsiella pneumoniae	Carbapenemase	NDM-1	64	32	32	32	32
Klebsiella oxytoca	Carbapenemase	KPC	> 64	> 32	> 32	16	16
Serratia marcescens	Carbapenemase	KPC	16	8	8	16	16
Acinetobacter baumanii	Carbapenemase	IMP-4	> 64	> 32	> 32	> 32	> 32
Acinetobacter baumanii	Carbapenemase	OXA-23 OXA-51	> 64	> 32	> 32	> 32	> 32
Pseudomonas aeruginosa	Carbapenemase	VIM-2	> 64	> 32	> 32	> 32	> 32
Escherichia coli	ESBL	TEM-26	> 64	4	0.06	< 0.03	0.25
Escherichia coli	ESBL	TEM-1 DHA	32	32	< 0.03	< 0.03	0.12
Escherichia coli	ESBL	SHV-2a	8	4	< 0.03	< 0.03	0.12
Escherichia coli	ESBL	TEM-1 CTX-M	> 64	> 32	0.12	0.12	0.25
Escherichia coli	ESBL	CTX-M	> 64	> 32	< 0.03	< 0.03	0.25
Escherichia coli	ESBL	TEM-19	4	4	< 0.03	< 0.03	0.25
Klebsiella pneumoniae	ESBL	SHV-11 CTX-M	> 64	> 32	4	0.06	0.25
Klebsiella pneumoniae	ESBL	SHV-18	> 64	8	0.06	0.06	0.12
Klebsiella pneumoniae	ESBL	SHV-5	0.5	0.06	< 0.03	0.06	0.5
Citrobacter freundii	AmpC	CMY-2	0.25	0.12	< 0.03	< 0.03	0.5
Escherichia coli	AmpC	TEM-1 CMY-2	64	8	0.06	< 0.03	0.5
Escherichia coli	AmpC	CMY-2	> 64	16	0.12	0.06	0.5
Klebsiella pneumoniae	AmpC	SHV-1 CMY-2	> 64	16	0.25	0.06	0.5
Klebsiella pneumoniae	AmpC	SHV-1 FOX	> 64	16	0.06	0.06	0.12
Morganella morganii	AmpC	DHA	< 0.06	0.06	0.06	0.25	4
Proteus mirabilis	AmpC	CMY-2	8	8	1	1	4
Proteus mirabilis	AmpC	CMY-2	64	32	0.5	4	32
Escherichia coli	None	None	1	0.06	< 0.03	< 0.03	0.25

CHROMagar KPC had a sensitivity (Se)/specificity (Sp) of 71.3%/100.0% for the detection of CPGNR whereas ChromID ESBL had a Se/Sp of 90.0%/45.0%.

TABLE 2
Sensitivity and specificity of CHROMagar KPC and ChromID ESBL for the detection of carbapenemase producing Gram negative bacilli

	CHROMagar KPC		ChromID ESBL	
Dilutions (CFU/mL)	**Sensitivity (%)**	**Specificity (%)**	**Sensibility (%)**	**Specificity (%)**
10^1	60.0	100.0	90.0	47.4
10^2	75.0	100.0	90.0	47.4
10^3	75.0	100.0	90.0	47.4
10^4	75.0	100.0	90.0	42.1
All dilutions	**71.3**	**100.0**	**90.0**	**46.1**

For carbapenem disks testing on MacConkey agar, the optimal inhibition zone diameters for screening of CPGNR were ≤ 24 mm for ertapenem, ≤ 34 mm for meropenem and ≤ 32 mm for imipenem

FIGURE 1
ROC curve for zones of inhibition around a 10-µg ertapenem disk for 39 challenge strains at different dilutions (10^1,10^2,10^3 and 10^4 CFU/mL)

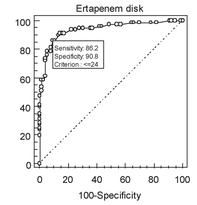


FIGURE 2
ROC curve for zones of inhibition around a 10-µg meropenem disk for 39 challenge strains at different dilutions (10^1,10^2,10^3 and 10^4 CFU/mL)

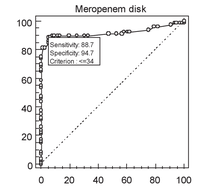
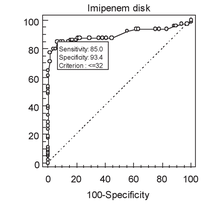


FIGURE 3
ROC curve for zones of inhibition around a 10-µg imipenem disk for 39 challenge strains at different dilutions (10^1,10^2,10^3 and 10^4 CFU/mL)



Using a detection threshold of 10^2 CFU/mL, the carbapenem disks had the following Se/Sp profiles: ertapenem 92.5%/92.1%, meropenem 95.0%/94.7% and imipenem100.0%/95.0%.

TABLE 3
Sensitivity and specificity of zone diameters around a 10-µg ertapenem (≤ 24 mm), meropenem (≤ 34 mm) and imipenem (≤ 32 mm) disk for detection of carbapenemase producing Gram negative bacilli

	Ertapenem		Meropenem		Imipenem	
Dilution (CFU/mL)	**Sen (%)**	**Spe (%)**	**Sen (%)**	**Spe (%)**	**Sen (%)**	**Spe (%)**
10^1	55.0	89.5	52.5	94.7	40.0	100.0
10^2	**92.5**	**92.1**	**95.0**	**94.7**	**100.0**	**94.7**
10^3	**100.0**	**89.5**	**100.0**	**94.7**	**100.0**	**89.5**
10^4	**100.0**	**84.2**	**100.0**	**94.7**	**100.0**	**89.5**
All dilutions	86.3	90.8	88.8	94.7	85.0	93.4

Conclusions

Screening stool for CPGNR using chromogenic media (CHROMagar KPC and ChromID ESBL) or the direct carbapenem disk method are reliable and easy to perform techniques. When comparing the two chromogenic media, the use of CHROMagar KPC would be better suited to detect CPGNR because of its high specificity compared to ChromID ESBL which has a very low specificity and would generate a high proportion of false positive results. Of concern is the fact that CHROMagar KPC failed to detect CPGNR with low MICs to ertapenem. Chromogenic media are expensive and require specific storage conditions therefore this should be kept in mind before choosing either CHROMagar KPC or ChromID ESBL as a method for CPGNR screening. The carbapenem disk method is inexpensive and has a good sensitivity and specificity at bacterial inoculum concentrations of 10^2 CFU/mL and over. Actually, the main limitation of this method is its poor sensitivity when a carbapenemase-producing bacteria is present at a low concentration (10^1 CFU/mL). To maximize the likelihood of finding CPGNR, two carbapenem disks per plate could be used. The performance characteristics of the chromogenic media as well as the carbapenem disk method are reliable and our results suggest that either one can be a useful tool for screening CPGNR in stool.

Acknowledgments

The plates of chromogenic media CHROMagar KPC (Alere Inc, Canada) and ChromID ESBL (BioMerieux, France) were kindly provided by the manufacturers.

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