

Comparison of Two Chromogenic Media and MacConkey Agar with Carbapenem Disks For the Detection of Carbapenemase-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 options¹. 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 spread². 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 swabs³⁻⁵. 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 agar⁶. 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 laboratories⁷⁻⁸. 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 specimens⁹⁻¹⁰⁻¹¹⁻¹². 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 specimens¹³. 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 cefodoxime (BioMérieux, Marcy l'Etoile, 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 MacConkey 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 10⁴ to 10¹ CFU/ml for each strain. The fecal inoculum was deposited 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 placed onto MacConkey agar. All media were incubated aerobically at 35°C for 24 hours. The plates were examined for growth and, if present, 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 (µg/ml)				
			CAZ	CTX	ERTA	MERO	IMI
<i>Citrobacter freundii</i>	Carbapenemase	KPC	> 64	> 32	16	16	16
<i>Enterobacter cloacae</i>	Carbapenemase	NMC	0.5	1	16	16	32
<i>Enterobacter cloacae</i>	Carbapenemase	KPC	> 64	> 32	8	4	4
<i>Escherichia coli</i>	Carbapenemase	KPC	> 64	> 32	8	4	16
<i>Escherichia coli</i>	Carbapenemase	KPC	> 64	> 32	4	4	4
<i>Escherichia coli</i>	Carbapenemase	KPC	> 64	> 32	4	4	8
<i>Escherichia coli</i>	Carbapenemase	KPC	> 64	> 32	4	4	16
<i>Klebsiella pneumoniae</i>	Carbapenemase	KPC	> 64	> 32	> 32	> 32	> 32
<i>Klebsiella pneumoniae</i>	Carbapenemase	KPC	> 64	> 32	> 32	> 32	> 32
<i>Klebsiella pneumoniae</i>	Carbapenemase	KPC	> 64	> 32	> 32	> 32	> 32
<i>Klebsiella pneumoniae</i>	Carbapenemase	KPC	> 64	> 32	> 32	> 32	> 32
<i>Klebsiella pneumoniae</i>	Carbapenemase	KPC	> 64	> 32	> 32	> 32	> 32
<i>Klebsiella pneumoniae</i>	Carbapenemase	OXA-48	1	1	4	2	8
<i>Klebsiella pneumoniae</i>	Carbapenemase	NDM-1	> 64	> 32	> 32	> 32	> 32
<i>Klebsiella pneumoniae</i>	Carbapenemase	NDM-1	64	32	32	32	32
<i>Klebsiella oxytoca</i>	Carbapenemase	KPC	> 64	> 32	> 32	16	16
<i>Serratia marcescens</i>	Carbapenemase	KPC	16	8	8	16	16
<i>Acinetobacter baumannii</i>	Carbapenemase	IMP-4	> 64	> 32	> 32	> 32	> 32
<i>Acinetobacter baumannii</i>	Carbapenemase	OXA-23	> 64	> 32	> 32	> 32	> 32
<i>Acinetobacter baumannii</i>	Carbapenemase	OXA-51	> 64	> 32	> 32	> 32	> 32
<i>Pseudomonas aeruginosa</i>	Carbapenemase	VIM-2	> 64	> 32	> 32	> 32	> 32
<i>Escherichia coli</i>	ESBL	TEM-26	> 64	4	0.06	< 0.03	0.25
<i>Escherichia coli</i>	ESBL	TEM-1	DHA	32	< 0.03	< 0.03	0.12
<i>Escherichia coli</i>	ESBL	SHV-2a	8	4	< 0.03	< 0.03	0.12
<i>Escherichia coli</i>	ESBL	TEM-1	CTX-M	> 64	> 32	0.12	0.12
<i>Escherichia coli</i>	ESBL	TEM-1	FOX	> 64	> 32	< 0.03	< 0.03
<i>Escherichia coli</i>	ESBL	SHV-11	CTX-M	> 64	> 32	4	0.06
<i>Klebsiella pneumoniae</i>	ESBL	SHV-18	> 64	8	0.06	0.06	0.12
<i>Klebsiella pneumoniae</i>	ESBL	SHV-5	0.5	0.06	< 0.03	0.06	0.5
<i>Citrobacter freundii</i>	AmpC	CMY-2	0.25	0.12	< 0.03	< 0.03	0.5
<i>Escherichia coli</i>	AmpC	TEM-1	CMY-2	64	8	0.06	< 0.03
<i>Escherichia coli</i>	AmpC	CMY-2	> 64	16	0.12	0.06	0.5
<i>Klebsiella pneumoniae</i>	AmpC	SHV-1	> 64	16	0.25	0.06	0.5
<i>Klebsiella pneumoniae</i>	AmpC	SHV-1	FOX	> 64	16	0.06	0.06
<i>Morganella morganii</i>	AmpC	DHA	< 0.06	0.06	0.06	0.25	4
<i>Proteus mirabilis</i>	AmpC	CMY-2	8	8	1	1	4
<i>Proteus mirabilis</i>	AmpC	CMY-2	64	32	0.5	4	32
<i>Escherichia coli</i>	None	None	None	1	0.06	< 0.03	< 0.03

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

Dilutions (CFU/ml)	CHROMagar KPC		ChromID ESBL	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	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 (10¹, 10², 10³ and 10⁴ CFU/ml)

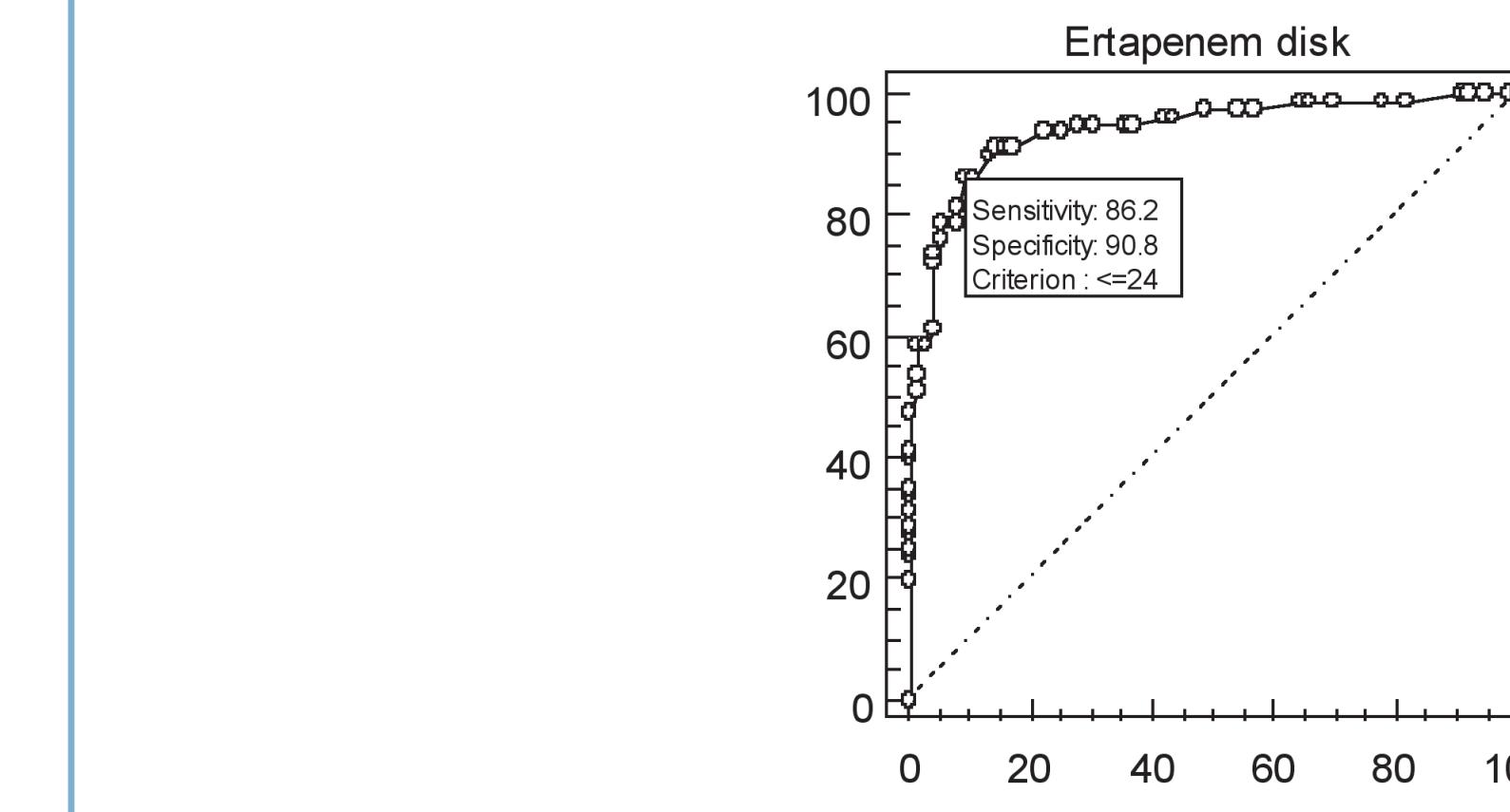


FIGURE 2
ROC curve for zones of inhibition around a 10-µg meropenem disk for 39 challenge strains at different dilutions (10¹, 10², 10³ and 10⁴ CFU/ml)

