Comparative evaluation of the performance of selective media for the detection of carbapenemase-producing *Enterobacteriaceae* isolates

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RICAI 2012 — 32e Réunion interdisciplinaire de chimiothérapie anti-infectieuse

No 280

Revised abstract

Aims

Early detection by screening of infected patients and carriers of carbapenemase-producing *Enterobacteriaceae* (CPE) is of utmost importance to prevent the spread of these isolates. We evaluated the performance of three selective media to detect CPE isolates.

Methods

A collection of 100 characterized CPE clinical isolates (OXA-48 [n=58], VIM-type [n=20], KPC-2 [n=17], NDM-1 [n=5]), 53 carbapenem non-susceptible *Enterobacteriaceae* (CNSE) isolates not producing carbapenemase (likely porin deficient) isolates and 24 carbapenem susceptible *Enterobacteriaceae* (CSE) isolates was challenged against the different selective culture media. Two different inocula were used: 10^6 CFU/spot (high inoculum) for CSE isolates and 10^2 CFU/spot (low inoculum) for CPE and CNSE isolates. The media used for detection of CPE were: chromogenic Colorex KPC medium (CKPC; CHROMagar), chromogenic Brilliance CRE medium (CCRE, Oxoid) and Supercarba medium (CSC; prepared on site with components kindly provided by L. Poirel/P. Nordmann, CHU Bicêtre, Paris). A MacConkey agar (Oxoid) was used as growth control. The analytical performances of each individual medium for the detection of CPE were calculated based on growth of CPE isolates at low inoculum and of CSE isolates at high inoculum.

Results

Overall sensitivities of 48%, 86% and 97% with CKPC, CCRE and CSC respectively were found for the detection of the 100 CPE isolates. All KPC-producing isolates were detected on all three media. However, significant differences in sensitivities were observed with OXA-48 producers (n=58) between CKPC (31%), CCRE (88%) and CSC (100%). Three CPE isolates only (all VIM-1 producers) did not grow on CSC. The specificities obtained with CKPC, CCRE and CSC were of 39%, 40% and 35% respectively for the inhibition growth of non-CPE isolates (n=77), but increased to 96%, 83% and 88% respectively when only CSE isolates (n=24) were considered.

Conclusions

CSC and CCRE displayed higher sensitivities compared to CKPC for the detection with low inocula of CPE isolates particularly for OXA-48 producers. CSC showed the best performance globally, but the additional workload required for the preparation of this medium may constitute a barrier for its use in a routine setting.

Introduction

• Rapid emergence and widespread of carbapenemase-producing *Enterobacteriaceae* (CPE) have been reported worldwide. [1, 2] Colonization or infections caused by CPE isolates represent a major public health threat for the individual therapeutic management and for the collective infection control issues.

• Adequate preventive measures for containing the spread of these multidrug resistant organisms are recommended and include active surveillance using appropriate screening methods for the detection of CPE isolates. [3, 4]

• This study aimed to compare the performance of three selective media for the detection of putative CPE isolates collected from hospitals throughout Belgium and referred to the National Reference Center for confirmation of their resistance mechanisms.

Methods

• **Bacterial isolates**:

  • A panel of 177 *Enterobacteriaceae* clinical isolates including 100 CPE (Table 1), 53 carbapenem non-susceptible *Enterobacteriaceae* (CNSE; probable porin deficiency) and 24 carbapenem susceptible *Enterobacteriaceae* (CSE).

  • MICs of the CPE isolates to ertapenem, imipenem and to meropenem were determined by broth microdilution using Sensititre BEGN panels (Trek Diagnostics System).

• **Agar plates**:

  • Three selective media were evaluated: Colorex™ KPC (CKPC; CHROMagar), chromogenic *Brilliance*™ CRE (CCRE; Oxoid) and Supercarba medium (CSC; Bicêtre, Pr. P. Nordmann) were evaluated. CSC plates were prepared on site with provided lyophilized components together with dehydrated Drigalski medium (Oxoid) [5].

  • MacConkey agar plates (MC; Oxoid) were used as growth control.

• **Culture procedures**:

  • Isolate stored at -80°C were thawed and subcultured twice on non-selective agar plate before plating on selective media.

  • Colonies of fresh pure culture of the isolate were suspended in saline water and adjusted to the density of 0.5 McFarland (± 10^8 CFU/ml).

  • For CSE isolates, a 10-µl aliquot of an undiluted 0.5 McF suspension was inoculated on the agar plates (high inoculum of 10^6 CFU/spot) .

  • For CPE and for CNSE isolates, the suspension is serially diluted at 1/10 down to 10^3 CFU/ml. 100 µl of the 10^3 CFU/ml concentration was inoculated on the agar plates (low inoculum of 100 CFU/spot).

  • All cultures were incubated for 24h at 35°C in normal atmosphere before reading.

• **Culture reading and results analysis**:

  • Interpretation of the culture results was performed according to the manufacturers' instructions. MacConkey agar plates were used as growth control indicator. Chromogenic features of the colonies were recorded. Quantification of growth was performed by colony counts on agar plates. For CPE isolates showing no growth on 10^2-dilution plate, culture of several serial 10-fold dilutions was performed by inoculating each dilution onto agar plates in order to determine the limit of detection (LOD).

  • Sensitivity and specificity of each medium was calculated by comparing growth to the genotypic characteristics of the tested strains (presence or absence of carbapenemase encoding genes).

Results

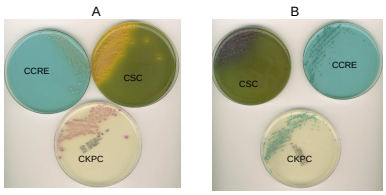
Table 1. Species, resistance mechanisms and growth on selective media of the CPE isolates (n=100)

		Carbapenemase coding gene				Growth on CKPC		Growth on CCRE		Growth on CSC	
Species	Total	OXA-48	VIM-type	KPC-2	NDM-1	Yes	No	Yes	No	Yes	No
K. pneumoniae	68	40	10	17	1	33	35	64	4	67	1
E. cloacae	16	12	3		1	9	7	11	5	16	
E. coli	7	4	1		2	1	6	4	3	6	1
K. oxytoca	3	1	2			2	1	3		3	
S. marcescens	2		2			1	1	2		2	
C. freundii	1	1				1		1		1	
C. braakii	1		1				1	1			1
M. morganii	1				1		1		1	1	
P. rettgeri	1		1			1			1	1	
Total	100	58	20	17	5	**48**	52	**86**	14	**97**	3

Table 2. Growth at low inoculum of 10^2 CFU/spot on the three selective media according to the resistance mechanisms (n=100 CPE isolates)

Number of CPE isolates		Growth on CKPC			Growth on CCRE			Growth on CSC		
Carbapenemase	Total	Yes	No	Sensitivity	Yes	No	Sensitivity	Yes	No	Sensitivity
OXA-48	58	18	40	31%	51	7	88%	58		100%
VIM-type	20	12	8	60%	15	5	75%	17	3	85%
KPC-2	17	17		100%	17		100%	17		100%
NDM-1	5	1	4	20%	3	2	60%	5		100%
Total	100	48	52	**48%**	86	14	**86%**	97	3	**97%**

Figures 1A &1B. Images of growth on selective media for a NDM-1 producing *E. coli* (A) and for a VIM-1 producing *S. marcescens* (B)



• Chromogenic features for CKPC/CCRE: All colonies that grew on either two media had adequate color except one VIM-1 positive *Providencia rettgeri* isolate colorless on CKPC (and not growing on CCRE)

Table 3. Growth at different inoculum size (limit of detection; LOD) on the three selective media in relation to the different types of CPE isolates (n=100)

		Type of CPE				
Media	LOD (CFU/ml)	OXA-48	VIM-type	KPC-2	NDM-1	Total
CKPC	<=10e3	18	12	17	1	48
	10e3-10e4	4	4		2	10
	10e4-10e5	4	1			5
	10e5-10e6	10	2		2	14
	10e6-10e7	13				13
	>10e7	9	1			10
CCRE	<=10e3	51	15	17	3	86
	10e3-10e4		3		2	5
	10e5-10e6		1			1
	>10e7	7	1			8
CSC	<=10e3	58	17	17	5	97
	10e3-10e4		1			1
	10e4-10e5		1			1
	10e6-10e7		1			1
Total		58	20	17	5	100

Table 4. Growth at high inoculum of 10^6 CFU/spot on the three selective media according to β-lactams resistance mechanisms of non-CPE isolates (n=77)

Number of non CPE isolates			Growth on CKPC		Growth on CCRE		Growth on CSC	
Carbapenem susceptible	Resistance mechanism	Total	Yes	No	Yes	No	Yes	No
No (CNSE)	Porin deficiencies	53	46	7	42	11	47	6
Yes (CSE)	ESBL	12	1	11	2	10	2	10
	AmpC	2		2	1	1		2
	DHA-1	1		1	1			1
	CMY-2	1		1		1	1	
	K-OXY	1		1		1		1
	OXA-1	1		1		1		1
	TEM-1	1		1		1		1
	Wild-type	5		5		5		5
	Total CSE	24	1	23	4	20	3	21
Total non CPE		77	47	30	46	31	50	27
Specificity	CNSE excluded			96%		83%		88%
	overall			39%		40%		35%

• No clear correlation could be found between CPE isolates carbapenem MICs and the growth rate on the different selective media

Conclusions

➢ Supercarba medium and *Brilliance*™ CRE showed significantly higher sensitivities as compared to Colorex™ KPC ($p<0.001$) for the detection of low inocula of CPE isolates particularly for OXA-48 producers.

➢ All three media displayed acceptable inhibiting effect against CSE isolates, but failed to differentiate carbapenemase producers from CNSE isolates with multiple β-lactams resistance mechanisms (ESBL/AmpC) associated with porin deficiency.

➢ Supercarba medium showed the best performance globally, but the extra- workload required for the home-made preparation of this medium may constitute a barrier for its use in a routine setting.

➢ Further studies are warranted to evaluate the performance of these selective media for the detection of CPE isolates on clinical or screening samples.

References

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