EVALUATION OF BRILLIANCE CRE AGAR FOR THE DETECTION OF CARBAPENEM-RESISTANT GRAM-NEGATIVE BACTERIA

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Background

Infections caused by carbapenem-resistant Enterobacteria are an emerging problem worldwide and a serious danger, especially for patients who require long-term hospitalisation (Rice *et al.*, 2009). Since the use of carbapenems has became predominant as a second or third-line drugs against multidrug-resistant Gram-negative bacteria, resistance to this class of molecules poses a serious problem in the management of nosocomial infections.

Resistance to carbapenems in Enterobacteriaceae can be mediated by different mechanisms: i) production of extended-spectrum beta-lactamases (ESBLs) associated with decreased membrane permeability (Jacoby *et al.*, 2004); ii) enzymes that are able to hydrolyse carbapenems such as metallo-beta-lactamases (MBLs) and the recently emerged class A carbapenemases (Queenan *et al.*, 2007). The most common enzymes in the last group are *Klebsiella pneumoniae* carbapenemases (KPC), which have been reported mostly in *Klebsiella pneumoniae* and are frequently found on mobile genetic elements with the consequent potential to spread widely (Nordmann *et al.*, 2009). Also, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, common agents of nosocomial infections, often show an MDR phenotype characterized by multiple drug resistance, including carbapenems.

Due to the rapid spread of these Gram-negative resistant bacteria and the scarcity of treatment options, it is essential to understand the mechanism of resistance in order to set up an adequate therapy and to start with appropriate infection control policies (Stuart et al., 2010). Various selective agar media have been developed for preliminary screening allowing different carbapenem-resistant enterobacterial species to be recognised easily (Adler *et al.*, 2011).

Methods

A total of 70 clinical isolates were studied. Of these, 30 were well-characterized carbapenem-resistant enterobacterial strains (Table 1), including *Klebsiella pneumoniae* producing KPC-type (n=15) or VIM-type (n=3) enzymes, VIM-positive *Enterobacter cloacae* (n=3) and *Escherichia coli* (n=1), and isolates characterized by porin loss associated with ESBL production (*K. pneumoniae*, n=3), or AmpC hyperproduction (*E. coli*, n=2; *Serratia marcescens*, n=2; *E. cloacae*, n=1). Ten additional carbapenemase-producing non-fermentative isolates (*Pseudomonas aeruginosa*, n=7; and *Acinetobacter baumannii*, n=3) were also included in the study as well as 30 carbapenem-susceptible Gram-negative isolates.

All isolates, stored at -80°C, were inoculated on Columbia Agar with Sheep Blood (Oxoid) with a 10 µg ertapenem disc. After 18-24 h incubation at 36°C, the colonies were inoculated on MacConkey (Oxoid) with a 10 µg ertapenem disc and plates were incubated aerobically at 36°C for 18-24 h. For each isolate MICs (Table 1) for ertapenem (ERT), imipenem (IMP), and meropenem (MEM) were determinated with Etest (bioMérieux, Marcy l'Etoile, France) and results were interpreted according to the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2011). Then a 0,5 MacFarland suspension of each isolate was prepared in sterile 0,9% saline (corresponding to a cell density of 1,5 x 10^8 CFU/ml) followed by further seven 10-fold serial dilutions (from 1,5 x 10^7 CFU/ml to 1,5 x 10 CFU/ml). Finally, 100 µl of each carbapenem-resistant strain were inoculated at three different concentrations onto Brilliance™ CRE Agar (1.5 x 10 CFU/ml, 1.5 x 10^2 CFU/ml, 1.5 x 10^4 CFU/ml), whereas 100 µl of each carbapenem-susceptible isolate were inoculated at the fixed dilution of 1.5 x 10^2 CFU/ml.

After 18-24 h incubation at 36°C the plates were evaluated in order to verify number, size and colour of colonies, and to determinate the level of detection (Table 1). All media were provided by Thermo Fisher Scientific.

Results

Data about number, size and colour of colonies were collected for all isolates after an overnight incubation. The Brilliance CRE Agar was consistently able to sustain the growth of carbapenem-resistant isolates, showing a detection limit of 1.5 x 10 CFU/ml in 31/40 cases whereas the remaining grew at 1.5 x 10^2 CFU/ml.

No growth was observed with carbapenem-sensitive control strains.

Colony size was defined as follows: large colonies (4-5 mm diameter), medium colonies (3 mm diameter), and small colonies (1-2 mm diameter). *K. pneumoniae* and *P. aeruginosa* grew with different colony sizes, *E. coli* showed both medium and large colonies, while in the case of *E. cloacae*, *S. marcescens* and *A. baumannii* we observed only a single type of colonies, medium for *E. cloacae* and small for the other ones, respectively.

Regarding the colour, blue colonies were obtained in the case of *K. pneumoniae* (Figure 1), *E. cloacae*, and *S. marcescens* whereas a pale pink colour was observed for *E. coli* (Figure 2). In particular, the colonies of *E. cloacae* and *S. marcescens* had sea green colour, distinguishable in most cases from steel blue of *K. pneumoniae*. Finally, *P. aeruginosa* showed a light brown colour (Figure 3) and *A. baumannii* grew with small colourless colonies (Figure 4).

Figure 1: *K. pneumoniae* producing KPC

Figure 2: *E. coli* producing VIM-1

Figure 3: *P. aeruginosa* producing VIM-1

Figure 4: *A. baumannii* producing OXA-23

Table 1. Level of detection on *Brilliance*™ CRE Agar, sensitivity, and resistance mechanism for the 30 CRE

SPECIES	LEVEL OF DETECTION (CFU/ml)	MIC (mg/L)			RESISTANCE MECHANISM
		ERT	IMP	MEM	
K. pneumoniae	1.5 x 10^2	16	8	8	KPC
K. pneumoniae	1.5 x 10^1	8	8	4	KPC
K. pneumoniae	1.5 x 10^2	16	16	16	KPC
K. pneumoniae	1.5 x 10^1	12	16	8	KPC
K. pneumoniae	1.5 x 10^1	> 32	24	4	KPC
K. pneumoniae	1.5 x 10^2	> 32	1	8	KPC
K. pneumoniae	1.5 x 10^2	12	16	12	KPC
K. pneumoniae	1.5 x 10^1	> 32	8	16	KPC
K. pneumoniae	1.5 x 10^2	16	8	8	KPC
K. pneumoniae	1.5 x 10^1	> 32	> 32	> 32	KPC
K. pneumoniae	1.5 x 10^1	> 32	8	2	KPC
K. pneumoniae	1.5 x 10^1	> 32	8	4	KPC
K. pneumoniae	1.5 x 10^2	> 32	16	> 32	KPC
K. pneumoniae	1.5 x 10^1	> 32	2	4	KPC
K. pneumoniae	1.5 x 10^1	> 32	1	2	KPC
K. pneumoniae	1.5 x 10^1	> 32	> 32	> 32	VIM-1
K. pneumoniae	1.5 x 10^2	> 32	> 32	> 32	VIM-1
K. pneumoniae	1.5 x 10^1	4	2	1	VIM-1
K. pneumoniae	1.5 x 10^1	8	0.19	1	CTX-M-15+porin loss
K. pneumoniae	1.5 x 10^2	8	0.19	2	CTX-M-15+porin loss
K. pneumoniae	1.5 x 10^1	16	0.125	1	CTX-M-15+porin loss
E. cloacae	1.5 x 10^1	8	4	2	VIM-1
E. cloacae	1.5 x 10^1	8	16	4	VIM-1
E. cloacae	1.5 x 10^1	8	32	2	VIM-4+SHV-12
E. cloacae	1.5 x 10^1	2	0.25	0.19	AmpC+porin loss
E. coli	1.5 x 10^1	2	4	0.75	VIM-1
E. coli	1.5 x 10^1	2	0.25	0.19	AmpC+porin loss
E. coli	1.5 x 10^1	2	0.25	0.25	AmpC+porin loss
S. marcescens	1.5 x 10^1	0.5	4	0.125	AmpC+porin loss
S. marcescens	1.5 x 10^1	0.5	2	0.125	AmpC+porin loss
P. aeruginosa	1.5 x 10^1	> 32	> 32	> 32	VIM-1
P. aeruginosa	1.5 x 10^1	> 32	> 32	> 32	VIM-2+PER-1
P. aeruginosa	1.5 x 10^1	> 32	> 32	> 32	VIM-1
P. aeruginosa	1.5 x 10^1	> 32	> 32	> 32	VIM-1
P. aeruginosa	1.5 x 10^1	> 32	> 32	> 32	VIM-1
P. aeruginosa	1.5 x 10^2	> 32	> 32	> 32	IMP-2
P. aeruginosa	1.5 x 10^1	> 32	> 32	> 32	IMP-13
A. baumannii	1.5 x 10^1	> 32	> 32	> 32	OXA-23
A. baumannii	1.5 x 10^1	> 32	> 32	> 32	OXA-24
A. baumannii	1.5 x 10^1	8	1	1	OXA-58

Conclusions

Our data demonstrates that the new Brilliance CRE Agar allows the growth of carbapenem-resistant isolates with low detection limits thus representing a useful screening medium for carbapenem-resistant enterobacteria. In our experience, carbapenem-resistant *P. aeruginosa* and *A. baumannii* were clearly distinguishable from enterobacterial strains based on difference in size and colour.

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