Comparison and development of faecal screening methods for detection of carbapenemase producing Gram negative bacteria.

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Introduction

Carbapenems are the ultimate drug choice for treatment of serious Gram negative infections in many hospitals. Increasing reports of bacteria producing carbapenemases, such as NDM, especially in outbreak scenarios, are of concern. Faecal screening policies for at risk patients are now commonplace. However the method for performing faecal screening accurately and for detecting carbapenemase producing bacteria is difficult. Commercial screening agars are available and official guidance has been issued but the issue remains problematic, especially in carbapenamase producing bacteria which exhibit low MICs to carbapenems. Here we compare the ability of commercial agars and official guidance to detect a variety of carbapenemase producing bacteria (CPB) at various concentrations within a faecal bacterial mix.

Methods

Commercial agars CRE (Oxoid) & ChromagarKPC (E&O) plus in-house agars containing MacConkey (MAC) + 8mg/L vancomycin (V) + 1mg/L meropenem (M) and UTI agar + 8mg/L V + 1mg/L ertapenem (ERT) were compared with a standard UTI agar (Oxoid) and HPA recommended MAC+ERT disc. CPBs at 10^9, 10^7, 10^5 & 10^3 cfu/mL were added to mixes of 10^9 *Pseudomonas aeruginosa* (PSA) or E. coli (EC) plus 10^7 *Enterococcus* sp (E). CPB used were: *Klebsiella pneumonia* (KPN) containing NDM (low MIC), E. cloacae (ECL) + NDM, *Acinetobacter baumannii* (AcB) + NDM (high MIC), PSA + VIM (high MIC), PSA + VIM (low MIC), KPN + IMP, KPN+ KPC, AcB + GES, KPN + oxacillinase (OXA) (Table 1).

Table 1: Faecal bacterial mix simulation

Mix No.	Background mix	CPB				IMI/MER MIC
		10^9	10^7	10^5	10^3	
1	10^9 PSA + 10^7 E	KPN NDM (L)				3 / 3
2	10^9 EC + 10^7 E	KPN NDM (L)				
3	10^9 PSA + 10^7 E	ECL NDM				8 / >32
4	10^9 EC + 10^7 E	ECL NDM				
5	10^9 PSA + 10^7 E	AcB NDM (H)				>32 / >32
6	10^9 EC + 10^7 E	AcB NDM (H)				
7	10^9 PSA + 10^7 E	PSA VIM (H)				>32 / >32
8	10^9 EC + 10^7 E	PSA VIM (H)				
9	10^9 PSA + 10^7 E	PSA VIM (L)				3 / 4
10	10^9 EC + 10^7 E	PSA VIM (L)				
11	10^9 PSA + 10^7 E	KPN IMP				32 / 32
12	10^9 EC + 10^7 E	KPN IMP				
13	10^9 PSA + 10^7 E	KPN KPC				>32 / >32
14	10^9 EC + 10^7 E	KPN KPC				
15	10^9 PSA + 10^7 E	AcB GES				>32 / >32
16	10^9 EC + 10^7 E	AcB GES				
17	10^9 PSA + 10^7 E	KPN OXA-48				12 / >32
18	10^9 EC + 10^7 E	KPN OXA-48				

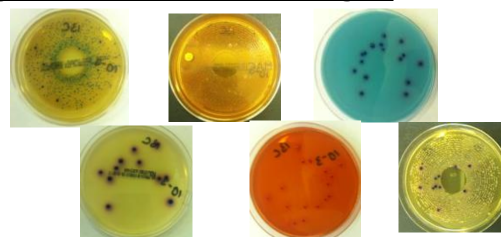
Table 2: Elimination of background & loss of inoculum

Mix No.	CRE		KPC		MAC+ERT disc		UTI+	
	Elimination of background	Log drop in CFU/mL	Elimination of background	Log drop in CFU/mL	Elimination of background	Detection by disc	Elimination of background	Log drop in CFU/mL
1	Y	3 (10^3)	X (PAER)	0	X (All)	Y	X (PAER)	0
2	Y	0	Y	0	X (All)		Y	0
3	Y	1 (10^3)	Y	1 (10^3)	X (All)	Y	X (PAER)	0
4	Y	0	Y	0	X (All)	Not at 10^3	Y	0.5 (10^3)
5	Y	0	X (PAER)	0	X (All)	Not at 10^3	Y	0
6	Y	0	X (Ent)	0	X (All)	Y	Y	0
7	Y	0	X (PAER)	0	X (All)	Y	Y	0
8	Y	0	Y	0	X (All)	Y	Y	0
10	Y	0	X (Ent)	0	X (All)	Y	Y	0
11	X (Ent)	0	X (Ent)	0.5 (10^5)	X (All)	Y	X (PAER)	0
12	Y	0	Y	1 (10^5)	X (All)	Not at 10^7, 10^5, 10^3	Y	0
13	X (Ent)	0	X (Ent)	0	X (All)	Not at 10^7, 10^5, 10^3	X (Ent)	0
15	X (Ent)	0	X (Ent)	0.5 (10^5, 10^3)	X (All)	Y	X (Ent)	0
16	Y	0	X (Ent)	1 (all)	X (All)	Y	X (Ent)	0.5 (10^7)
17	Y	0	X (Ent)	1 (all)	X (All)	Not at 10^7, 10^5	X (Ent)	0
18	Y	0	X (Ent)	1 (all)	X (All)	Not at 10^7, 10^5	Y	0

Results

For agars CRE & UTI+V+ERT all CPB were detected with no loss of quantity and ease of distinguishing against background bacteria was good for all mixes. For KPC & MAC+V+MER, CPB were detected with only slight loss of quantity in 10^5 and 10^3 quantities in mixes 15 to 18 (Table 2). For MAC+ERT disc, background isolates grew making ease of distinguishing CPB reasonable for mixes 1, 3, 4(10^9/10^7), 5(10^9-10^5), 6, 13(10^9/10^7), 15, 16, 17(10^9), & 18(10^9). At lower concentrations (10^5/10^3), ease of detection in mixes 4, 5, 12, 13, 17, 18 was poor and in mix 4 at 10^3 not detected at all.

Figure 1: Mix 13 on selective agars



Conclusions

CRE commercial plate and UTI+VAN+ERT performed best at detecting CPB and for ease of distinguishing from background mix. MAC + ERT disc performed variably depending on the quantity of CPB present.

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