Evaluation of various culture media and procedures recommended for isolation of *Enterobacteriaceae* that produce NDM-1 carbapenemase

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Introduction and purpose:

For many patients, carbapenems are a 'last resort' for treatment of multi-drug resistant *Enterobacteriaceae*. The increasing incidence of infections caused by carbapenemase-producing *Enterobacteriaceae* (CPE) is a major concern. Of particular concern to some, is the increasing occurrence of bacteria harboring NDM-1 enzyme, due to the genetic plasticity of the plasmid encoding it, and the relative ease of its transmission between different taxa.[1]

The UK Department of Health has issued guidance to minimize the risk of the dissemination of carbapenemases in UK hospitals.[2] This guidance includes a recommendation to screen specimens (including faeces or rectal swabs) from 'high-risk' patients on admission. Such patients include those with previous hospitalization or dialysis in countries where CPE are prevalent.

A number of procedures and/or culture media have been recommended for isolation of CPE and new chromogenic media designed for their isolation have been commercialised. We compared the performances of the available media along with other recommended methods for isolation of CPE.

Methods:

Challenge of media with pure cultures:

Sixty four previously identified NDM-1 producing *Enterobacteriaceae*[3] were inoculated at 10^5 and 10^2 colony forming units (CFU) per 1 µl spot onto media designed or recommended for isolation of CPE including:

- ✓**Brilliance CRE** (Oxoid).
- ✓**Colorex KPC** (E&O Laboratories).
- ✓**chromID ESBL** (bioMérieux; recommended for detection of CPE by Carrër *et al.*).[4]
- ✓**chromID CARBA** (a prototype medium provided by bioMérieux, based on the same principles of a previously evaluated medium, 'IDCARBA').[3]
- ✓**MacConkey agar plus 1 mg/l imipenem** (prepared in-house).

Tryptone Soya Broths (TSB) plus 2 mg/L ertapenem or 2 mg/L meropenem were also challenged with 7500 CFU of each isolate and then sub-cultured onto MacConkey agar (CDC recommended method for rectal swab screening). 5 ml volumes of TSB broth (Oxoid) were supplemented with a 10 µg disc of ertapenem or meropenem, as recommended.

Challenge of media with clinical samples:

100 local stool samples (Newcastle, UK) were also inoculated onto all media listed above. Finally, 327 stool samples referred to two Pakistan Public Sector Laboratories were cultured onto chromID CARBA and Brilliance CRE. All Gram-negatives isolated from the 427 stool samples on any of the media were investigated for carbapenemases, AmpC and ESBLs by phenotypic methods and/or genotypic methods as previously described.[3]

Results:

Table 1: Number of NDM-1 positive *Enterobacteriaceae* inhibited (i.e. 'false-negatives') at 10^2 and 10^5 cfu/spot on media recommended for the detection of CPE.

	n	chromID ESBL		chromID CARBA		Brilliance CRE		MacConkey / imipenem		Colorex KPC		TSB plus ertapenem	TSB plus meropenem
		10^2	10^5	10^2	10^5	10^2	10^5	10^2	10^5	10^2	10^5	7500 cfu / broth	
Citrobacter spp.	8	0	0	0	0	1	1	7	6	2	0	2	5
Escherichia coli	30	0	0	0	0	12	12	23	18	12	1	2	13
Enterobacter cloacae	21	0	0	0	0	0	0	20	5	1	0	1	3
Klebsiella pneumoniae	3	0	0	0	0	0	0	3	3	1	0	0	2
Providencia rettgeri	2	1	1	2	2	2	0	2	0	1	0	2	2
Sensitivity		**98%**	**98%**	**97%**	**97%**	**77%**	**80%**	**14%**	**47%**	**73%**	**98%**	**89%**	**61%**

Table 2: *Enterobacteriaceae* with carbapenemase, and other isolates, recovered from 100 local stool samples.

	Carbapenemase-producing *Enterobacteriaceae*						
	chromID ESBL	chromID CARBA	Brilliance CRE	MacConkey / imipenem	Colorex KPC	TSB plus ertapenem	TSB plus meropenem
Total:	0	0	0	0	0	0	0
Non-carbapenemase producers.							
Acinetobacter spp.	0	0	0	0	0	2	0
Pseudomonas spp.	12	9	2	13	3	15	5
S. maltophilia	0	0	2	0	0	0	1
Enterobacteriaceae	**22**	3	2	**14**	1	**39**	**43**
Candida spp.	0	0	0	3	0	5	12
Lactobacilli	0	0	0	0	0	0	1
Enterococcus spp.	3	2	0	28	0	**99**	**82**
Staphylococcus spp.	0	0	0	1	0	7	9
Streptococcus sp.	0	0	0	0	0	0	1
Total isolates	**37**	**14**	**6**	**59**	**4**	**167**	**155**

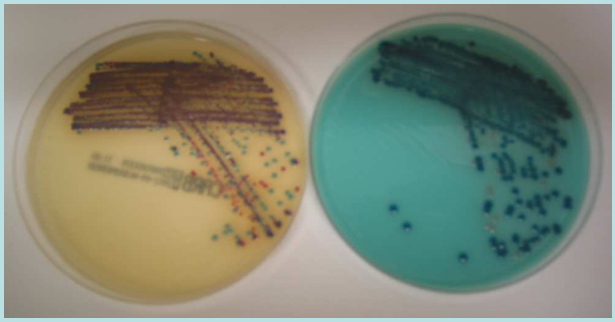

Fig. 1: *E. coli* (pink/red colonies) and *K. pneumoniae* (blue/green colonies) on chromID CARBA (left) and Brilliance CRE (right) isolated from a stool sample. Both species produced NDM-1 carbapenemase.

Table 3: Isolation of NDM-1 producing *Enterobacteriaceae* from 327 stool samples using two chromogenic media at two laboratories in Pakistan.

	National Institute of Health, Islamabad (*n* = 152)			Armed Forces Institute of Pathology, Rawalpindi (*n* = 175)		
NDM-1-POSITIVE	Both media	Brilliance CRE	chromID CARBA	Both media	Brilliance CRE	chromID CARBA
Klebsiella pneumoniae	5	4	4	10	7	10
Escherichia coli	8	3	8	21	12	20
Enterobacter cloacae	1	0	1	4	1	4
Citrobacter freundii	1	0	1	1	1	0
Kluyvera georgiana	1	1	0	-	-	-
Total isolates:	16	**7**	**14**	36	**21**	**34**
Sensitivity (%):		43%	88%		58%	94%
Total patients detected:	13	**7**	**12**	31	**20**	**31**
Sensitivity (%):		54%	92%		65%	100%
Carbapenemase- Negative *Enterobacteriaceae*	Both media	Brilliance CRE	chromID CARBA	Both media	Brilliance CRE	chromID CARBA
Total Isolates:	138	**135**	7	156	**153**	17

Conclusions:

When challenged with 64 NDM-1 producing *Enterobacteriaceae*, chromID ESBL and chromID CARBA both showed efficient recovery of the vast majority of isolates (97-98%) even at low inocula. For Colorex KPC and Brilliance CRE, approximately a quarter of isolates failed to grow at low inocula (10^2 cfu/spot). MacConkey plus 1 mg/L imipenem was inhibitory to at least half of the NDM-1 producers whereas TSB plus ertapenem was less inhibitory than TSB plus meropenem (Table 1).

No carbapenemase producers were isolated from 100 local stool samples (Newcastle, UK) using any medium, however there was a clear advantage of Colorex KPC, Brilliance CRE and chromID CARBA in terms of selectivity. In particular, cultures derived from TSB broths (containing either ertapenem or meropenem) frequently yielded a heavy growth of *Enterococcus* sp. (Table 2).

52 isolates of CPE were recovered from 44 of 327 patients in Pakistan (prevalence: 13.5%). All CPE produced NDM-1 enzyme and no other carbapenemase-types were detected. chromID CARBA showed a superior performance when compared with Brilliance CRE for isolation of NDM-1 producing *Enterobacteriaceae* from stool samples at two Pakistan Public Sector Laboratories. Significantly more patients colonized with NDM-1 producers were detected using chromID CARBA when compared with Brilliance CRE (43 *vs.* 27; $P = 0.0004$). This may have been due to the lack of selectivity of Brilliance CRE in this environment (Table 3) as large numbers of *Enterobacteriaceae* were recovered on this medium that showed no evidence of carbapenemase activity.

References:
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