P 0843 Evaluation of loop-mediated isothermal amplification (LAMP) assay for detection of carbapenem-resistant Enterobacteriaceae in cultures

Per Rydström and Håkan Janson
Department of Clinical Microbiology, Central Hospital, Växjö, Sweden

Introduction

The increasing prevalence of carbapenem resistant Enterobacteriaceae (CRE) poses an ever increasing problem in hospital settings and out-patient clinics all over the world. A rapid and reliable detection of CRE from cultures and rectal swabs can aid clinicians towards better treatment results and a more sensible use of antibiotics. The Amplex easyplex SuperBug® complete B provides a loop-mediated isothermal amplification (LAMP) detection assay of seven groups of carbapenemase genes for this purpose (table 1).

Materials and methods

Out of the 46 isolates tested, two were reference strains (see table 2), 18 were clinical isolates and 26 were isolates from our culture collection. The clinical isolates were chosen based on a phenotypic resistance in disc diffusion to ertapenem and/or where the meropenem zone fell below or was close (1 mm) to the recommended screening cut-off diameter (as per EUCASTs definition). All applicable and available clinical samples from 2014 and 2015 were included.

Samples from the culture collection were chosen to encompass as many of the available groups of carbapenemase genes included in the kit as possible. The carriership of any β-lactamase or carbapenemase gene(s) had previously been examined and was known for all strains, including clinical samples, but was hidden from the examiner at the time of analysis. No rectal swabs were tested.

Results

All known carbapenemase genes included in the assay were detected in all samples (32 samples). The most common carbapenemase gene groups were OXA-48 and NDM, in nine isolates respectively. KPC and VIM followed with seven isolates each and two isolates were positive for OXA-181.

The OXA-181 positive strains were also simultaneous carriers of a KPC-group gene. No strains with OXA-23 or OXA-40 were tested. The strains carrying carbapenemase genes from the IMI family and IMP-26 respectively were not detected (one sample each). All non-CRE strains were successfully classified as such (12 samples).

Of all strains, 22 were *Klebsiella pneumoniae* (48%), 15 were *Escherichia coli* (33%) and three *Enterobacter cloacae* (7%). The remainders were *Enterobacter aerogenes* (2; 4%), *Klebsiella oxytoca*, *Enterobacter asburiae*, *Proteus mirabilis* and *Citrobacter koseri* respectively (1; 2% each).

Conclusions

All strains of CRE were successfully detected (light green colour in table 2), excluding the IMI and IMP family of enzymes respectively (not included in the assay; orange colour). All non-CRE strains possessed other different classes of β-lactamases, including ESBL A and AmpC, which remained undetected with this assay (dark green colour).

For a lab of our relatively small size, assuming 100 assays used per year renders a cost of €84 per sample analyzed, including initial purchase, service costs and labor. Concluding, this method faithfully and rapidly, with minimal training and intervention, detects the most common groups of carbapenemase enzymes in Enterobacteriaceae in cultures, albeit at a high cost.

Table 1: Carbapenemase genes included in assay

Group	Specificity
KPC	KPC 1-15
NDM	NDM 1-7
OXA-48	OXA-48, -162, -204, -244
VIM	VIM 1-37
OXA-23	*e.g.* OXA-23, -27, -49, -73
OXA-40	*e.g.* OXA-24, -25, -26, -40, -72
OXA-58*	OXA-58, -96, -97
OXA-181**	OXA-181, -232

* A version, ** B version

Table 2: Results from Amplex easyplex SuperBug® complete B compared to reference testing

Species	Amplex	Reference	Species	Amplex	Reference
Klebsiella pneumoniae	NDM, OXA-181	NDM-1, OXA-181	*Escherichia coli*	VIM	VIM-1
Klebsiella pneumoniae	OXA-48	OXA-48	*Escherichia coli*	NDM	NDM-1
Klebsiella pneumoniae	-	ESBL A	*Escherichia coli*	-	IMP-26
Klebsiella pneumoniae	-	AmpC	*Escherichia coli*	OXA-48	OXA-48, CTX-M
Klebsiella pneumoniae	VIM	VIM	*Escherichia coli*	VIM	VIM
Klebsiella pneumoniae	VIM	VIM	*Escherichia coli*	NDM, OXA-181	NDM, OXA-181
Klebsiella pneumoniae	KPC	KPC-3	*Escherichia coli*	-	CTX-M, CIT
Klebsiella pneumoniae	OXA-48	OXA-48	*Escherichia coli*	OXA-48	OXA-48
Klebsiella pneumoniae	KPC	KPC-2	*Escherichia coli*	-	CIT
Klebsiella pneumoniae	KPC	KPC	*Escherichia coli*	OXA-48	OXA-48
Klebsiella pneumoniae	-	ESBL A	*Escherichia coli*	NDM	NDM
Klebsiella pneumoniae	OXA-48	OXA-48	*Escherichia coli*	OXA-48	OXA-48
Klebsiella pneumoniae	-	ESBL A	*Escherichia coli*	OXA-48	OXA-48
Klebsiella pneumoniae	-	ESBL A	*Escherichia coli***	-	CTX-M-15
Klebsiella pneumoniae	-	ESBL A	*Enterobacter cloacae*	KPC	KPC
Klebsiella pneumoniae	-	AmpC	*Enterobacter cloacae*	NDM	NDM
Klebsiella pneumoniae	KPC	KPC	*Enterobacter cloacae*	-	AmpC
Klebsiella pneumoniae	NDM	NDM-1	*Enterobacter aerogenes*	-	ESBL A
Klebsiella pneumoniae	VIM	VIM	*Enterobacter aerogenes*	NDM	NDM
Klebsiella pneumoniae	VIM	VIM	*Klebsiella oxytoca*	VIM	VIM
Klebsiella pneumoniae	KPC	KPC-3, TEM	*Enterobacter asburiae*	-	IMI
*Klebsiella pneumoniae**	KPC	KPC-2	*Citrobacter koseri*	NDM	NDM
Escherichia coli	OXA-48	OXA-48	*Proteus mirabilis*	NDM	NDM

* CCUG 56233, ** NCTC 13353; light green: assay true positive; dark green: assay true negative; orange: gene not included in assay

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