Evaluation of BD Phoenix™ CPO Detect Assay for Detection of Carbapenemase Producing Organisms in Clinical Samples in Singapore

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Abstract

Rapid and accurate detection of Carbapenemase Producing Organisms (CPO) is crucial to a targeted infection control strategy, as in Tan Tock Seng Hospital (TTSH), a large tertiary hospital in Singapore, where cohorting of CPO colonised patients is driven by PCR-based genotypic identification. A newly released panel for the BD Phoenix system, the CPO Detect panel, includes CPO detection with Ambler Class identification, alongside standard antibiotic susceptibility testing as with other Phoenix panels. We evaluated this system as compared to the Carbapenemase PCRs used at both TTSH and the National Public Health Laboratory (NPHL).

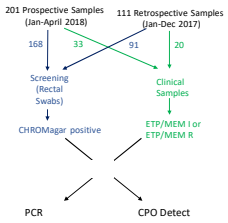
Introduction

The hospital management of CRO still relies heavily on physical separation of CPO colonized and CPO naive patients. The CRO control strategy at TTSH depends on i) screening through taking of rectal swabs on patient admission and culture on chromogenic media or ii) identifying clinical isolates (blood, urine or wound sources) exhibiting carbapenem resistance in antibiotic susceptibility testing. In both cases, PCR detecting the most common carbapenemases found in Singapore (KPC, NDM, IMI, IMP and OXA-48 sub-types) is used to confirm carbapenemase production and define CPO cohorts within the hospital. However, the process of growth on chromogenic agar, DNA extraction and subsequent PCR requires 2-3 days post-admission. Any decrease in the time taken to detect patients colonized with CPO could be expected to reduce the rate of CPO transmission in the hospital.

Phoenix CPO Detect™ is a novel gram negative diagnostic panel recently released by BD Diagnostics Systems for use with the Phoenix automated system. The panel tests for carbapenemase production with simultaneous Ambler classification, within 12-18 hours. We evaluated the CPO Detect™ assay in comparison with our standard in-house PCR.

Methods

All isolates undergoing routine CPO detection at TTSH between January and April 2018 were sampled prospectively. 201 prospective samples comprised 168 Rectal Swab isolates (with positive result on chromogenic agar) and 33 clinical isolates (highlighted due to Meropenem or Ertapenem non-susceptibility in antibiotic susceptibility testing). 101 samples were retrospectively taken from a frozen biobank of CPO positive samples from 2017. PCR was carried out on all samples using primers against KPC[1], IMI[2], IMP[1], NDM[1] and OXA-48[3].



All samples were assayed using CPO Detect in the Phoenix M50 system. Retrospective samples were subjected to a PCR developed at the NPHL which detects more carbapenemase subtypes.

[1] Teo *et al*, 2011. WPSAR; 3(1): 19-24. [2] Teo *et al*, 2013. JMM; 62: 1086-88 [3] Balm *et al*, 2011. JCM; 50(2): 475-6

	Clinical	Screening	Clinical	Screening	Total
Citrobacter amalonaticus		2		1	3
Citrobacter freundii	1	2	1	2	6
Citrobacter koseii		2		1	3
Enterobacter aerogenes		7		2	9
Enterobacter asburiae		3		1	4
Enterobacter cloacae		27	4	18	49
Enterobacter kobei				4	4
Escherichia coli	8	31	3	24	66
Escherichia vulneris		1		1	2
Klebsiella pneumonia	24	92	6	30	152
Morganella morganii				1	1
Proteus mirabilis			1		1
Serratia marcescens		1		1	2
Total	15	86	33	168	302

Fig. 1 - Isolates used in this study

Results

Detection of Carbapenemases by in-house PCR and CPO Detect

The CPO Detect Assay identified carbapenemase activity in 66% of the routine (prospective) samples assayed between Jan-April 2018 (Fig. 2), whereas the in-house TTSH PCR identified a specific carbapenemase (IMI, IMP, KPC, NDM or OXA-48) in 52% of the same samples.

PCR	CPO Detect: Carbapenem Detected	CPO Detect: Negative
Carbapenem Detected	96	10
Negative	37	58

201

Fig. 2 – Higher rates of CPO detection by CPO Detect than PCR

Agreement in Ambler Classification of by CPO Detect and in-house PCR

The CPO Detect Assay classifies CPO positive samples according to Ambler Class A, B or D, or where CPO activity is detected, but no Ambler class is specified*. Where ESBL activity was indicated, this was considered not to affect carbapenemase activity in our analysis.
Ambler classification by CPO Detect agreed with PCR results (including classification as CPO negative) in 142/201 samples (70.6%).
There were 9 isolates in which PCR amplified a specific carbapenemase, but which CPO Detect labelled as negative – these were considered as errors on the part of the CPO Detect assay. Of these, 7 were false negatives in IMI containing isolates (fig. 3A).
There were similarly 37 cases detected by CPO Detect, but negative by PCR and these were considered PCR inaccuracies.
Ambler classification matched PCR results in 84/88 cases where it was given by CPO Detect (95.5%). Partial agreement arose in cases where more than one carbapenemase was noted by PCR, or when CPO Detect did not specify an Ambler class.

A

PCR Result (Ambler Class)	CPO Detect Result: A	B	D	CPO Detected*	Atypical ESBL	ESBL	Neg	Total
IMI (A)	6						7	13
IMP (A)		1			1			2
KPC (A)	7					1		8
NDM (B)		17	3	2				22
OXA-48 (D)	1		53	1				55
OXA-48 and IMP			1					1
NDM and OXA-48		1	3		1			5
Negative	2	18	2	15	34	5	19	96
Total	16	35	62	18	36	6	28	201

B

		%
Agreement (CPO Detection)	142	70.6
Phoenix inaccuracy	13	6.5
PCR inaccuracy	37	18.4
Partial agreement	9	4.5
Agreement (Ambler Class)	84/88	95.5

Fig.3 - Comparison of carbapenemases identified by in-house PCR with Ambler class identified by CPO Detect (A) and agreement (B). Results are shaded according to complete agreement, partial agreement or disagreement.
* CPO Detected, but Ambler class not specified.

Identification of carbapenemases in selected, retrospective samples

To investigate CPO detection at a higher resolution, we selected isolates from our CPO biobank from the previous year, and compared CPO Detect results with a more discriminatory PCR developed at the NPHL. Noting that a large number of IMI genotypes had not been detected by the CPO Detect panel, we chose all isolates in our bank carrying the IMI genome. We also selected IMP and OXA genotypes which had been under-represented in our prospective sample as well as isolates known to carry more than one carbapenemase and those having carbapenem resistance due to carriage of AmpC with porin mutations.

Our results showed that CPO Detect appropriately detected carbapenemases activity in 91/95 isolates (95.7%). It failed to detect 3 IMI-1 containing isolates and one oxa-23 isolate (4.2%).

CPO Detect produced a positive result in 4 isolates, where PCR had been negative.
Ambler classification corresponded to the genome identified by PCR in 62 of 66 samples in which Ambler classification was given (93.9%), but CPO Detect remained unable to distinguish between those isolates carrying more than one carbapenemase. The lower success rate of CPO detection, as well as lower accuracy of Ambler classification was expected in our retrospective samples, since these were selected specifically to include genotypes CPO Detect had previously had difficulty.

A

NPHL PCR	Phoenix (Ambler Class): A	B	D	CPO Detected	Atypical ESBL	ESBL	Neg	Total
AmpC + porin/efflux		4		1	1			6
IMI-1	7						3	10
IMI-2	1							1
IMI-4	5							5
IMP-1	1	7						8
IMP-4		9		3				12
KPC	7	1		1				9
NDM		8						8
OXA-48		1	8	1				10
OXA-23						1		1
OXA-181		1	9					10
OXA-232			4	1				5
NDM and IMP-4		2						2
OXA-181 and NDM			5					5
OXA-23 and OXA-48			1					1
OXA-232 and NDM			1					1
OXA-48 and NDM		1	1					2
OXA-48 and IMP-10			1					1
Negative		2		2				4
Total	21	36	30	9	1	1	3	101

B

		%
Agreement	67	66.3
Phoenix inaccuracy	14	13.9
Partial agreement	20	19.8
	101	

Fig.4 - Comparison of carbapenemases identified by NPHL PCR with Ambler class identified by CPO Detect (A) and showing agreement between PCR and CPO Detect (B). Results are shaded as previously.

Conclusion

Our results show a higher rate of overall CPO detection by the Phoenix CPO Detect assay than our in-house PCR. This is not surprising, since the TTSH PCR is designed to detect the five most common carbapenemase genotypes in Singapore, whereas the CPO Detect panel has a more phenotypic determination of carbapenemase activity. The savings in time and manpower compared to PCR make CPO Detect a useful tool in CPO control efforts such as ours.

However, there are areas where CPO was inferior to PCR, such as the poor detection of IMI-1 and isolates carrying dual-carbapenemases were only partially classified.

Ambler classification by CPO Detect was less consistent with either PCR than straightforward CPO detection. While identifying the existence of CPOs is the primary aim of screening for carbapenemases, at TTSH we use genotype of identified CPOs to inform our epidemiological investigations of carbapenemase transmission.

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