A Rapid 3base™ Real-Time PCR for the detection of Extended Spectrum Beta-Lactamase and Carbapenemase Producing Organisms using Multi-Coloured Fluorogenic Probes

Damien J Stark[1], John Harkness[1], John R Melki[2] and Douglas S Millar[2]

[1] Microbiology Department, St.Vincent's Hospital, Darlinghurst, NSW 2010, [2] Genetic Signatures, Level 9, Lowy Packer Building, 405 Liverpool Street, Darlinghurst 2010

Introduction

Beta-lactam antibiotics are the most commonly used antibiotics worldwide in the treatment of bacterial infections. Extended Spectrum Beta-Lactamase (ESBL) producing organisms hydrolyse cephalosporins, penicillins and aztreonam rendering them ineffective for treatment of infected patients. Recently there have been reports of ESBL organisms expressing multiple drug resistance markers, making treatment of infected patients more challenging. ESBLs do not hydrolyse carbapenems thus carbapenems have become the first line choice in the treatment of ESBL infections. The emergence of carbapenemase resistant bacteria is a significant global concern in healthcare settings, as standard treatments may be rendered ineffective. Therefore, accurate and timely detection of ESBL and Carbapenemase Producing Organisms (CPO) will have a significantly impact on patient management.

We have developed a rapid real-time PCR (RT-PCR) assay to detect both ESBL and CPO (see Table 1). In order to produce a single tube assay using a standard 4-channel RT-PCR instrument we utilised multi-coloured fluorescent probes. This strategy increases the multiplex capability of a standard 4-channel instrument from four to ten and up to fifteen or more on a 5-channel instrument (see Figure 1).

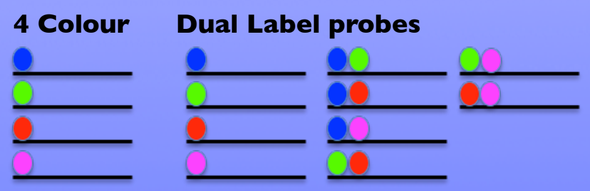

Figure 1. Increase in PCR multiplexing achieved when using dual label probes.

Table 1. Targets included in the Genetic Signatures ESBL/CPO assay.

Target	Types detected
Pan-TEM	>100 types
Pan-SHV	>50 types
Pan-CTX-M	Group 1 and group 9
Pan-CMY	2, 4, 6, 7, 14-16, 18, 21-35, 37-38, 40, 42-45, 49, 53-62, 69, 73
Pan-DHA	1, 2, 5-7, 9-10, 12-22, 24
Oxa-48	48, 48b, 162, 163, 204, 244, 245, 247, 370, 405
Oxa-181	181, 405, 416, 438, 505
NDM	1-12, 16
KPC	All types
VIM	1-7, 8-20, 23-28, 31, 33-44, 49-50
IMP	1-4, 6,7, 10, 14, 25-26, 28,30, 34, 40, 42, 52, 55

Methods/Materials

Nucleic acids were converted to a 3base™ form during the DNA isolation in order to yield better multiplexed PCR performance (www.geneticsignatures.com). The assay sensitivity was determined using synthetic DNA constructs and assay performance assessed using reference material from ATCC (Manassas, USA) and Zeptometrix (Buffalo, USA). Clinical performance was assessed using isolates obtained from St. Vincent's Hospital (Sydney, Australia). DNA extraction and PCR set up was performed on a GS1 automated extraction platform (Genetic Signatures, Sydney, Australia) resulting in a significant reduction in hands on time. PCR was performed on a 5 colour CFX real-time PCR instrument (Bio-Rad, California, USA) with integrated software calling.

Results

The sensitivity of each component in the final multiplexed assay was found to be less than 5 copies of target when introduced into the RT-PCR reaction. An example of real time traces generated are shown in figure 2. Mixed infections were easily detected using the multi-coloured probe approach, as they appear in different combination of colours and Ct values. The specificity of the assay was assessed on a cross reactivity panel which showed no cross reactivity. Results from the validation panel yielded 100% concordance with the expected resistance patterns.

Figure 2. Sensitivity of detection of NDM.

Table 2. Resistance genes detected using the Zeptometrix validation panel. *Not included in the Verigene BC-GN panel.

Panel member	Verigene BC-GN result	Genetic Signatures
P. aeruginosa	VIM	VIM
Providensia sp.	NDM	NDM
P. vulgaris		
K. pneumoniae	CTX-M, OXA	CTX-M, OXA-48, *TEM, *SHV
K. pneumoniae	CTX-M, IMP	CTX-M, IMP, *SHV
K. pneumoniae	KPC	KPC, *SHV, *TEM
K. oxytoca		
E. coli		
E. cloacae		
C. freundii		*CMY
A. baumannii		
Negative		

Table 3. Results obtained using clinical isolates

Sample#	Resistance genes detected	Sample#	Resistance genes detected	Sample#	Resistance genes detected
1	NDM/TEM/CTX-M/SHV/CMY	31	TEM	61	CMY
2	IMP/TEM	32	IMP/TEM/CTX-M/SHV/CMY	62	TEM/CTX-M/SHV
3	IMP/TEM/CTX-M/CMY	33	IMP/TEM	63	TEM/CTX-M
4	IMP/TEM/CTX-M	34	KPC/CTX-M/SHV/CMY	64	CTX-M
5	IMP/VIM/TEM	35	KPC/CTX-M/SHV/CMY	65	TEM/CTX-M
6	IMP/VIM/TEM/DHA	36	NDM/TEM/CTX-M/SHV/CMY	66	CTX-M
7	VIM	37	IMP/TEM/CTX-M/SHV/CMY	67	CTX-M
8	TEM/CTX-M	38	IMP/TEM	68	TEM/CTX-M
9	KPC/TEM/SHV/CTX-M/CMY	39	IMP/TEM	69	CTX-M
10	IMP/TEM/CTX-M/SHV/CMY	40	VIM	70	NEGATIVE
11	VIM/TEM	41	IMP/TEM/CTX-M/SHV/CMY	71	CTX-M
12	KPC/TEM/SHV/CTX/CMY	42	TEM/CTX-M/SHV/CMY	72	NEGATIVE
13	KPC/SHV/CTX-M/CMY	43	IMP/TEM/CTX-M/SHV/CMY	73	CTX-M
14	IMP/TEM	44	TEM/CTX-M/SHV/CMY	74	TEM/CTX-M
15	KPC/TEM/CTX-M/SHV/CMY	45	IMP/TEM/CTX-M	75	CTX-M
16	NDM/TEM/CTX-M/SHV/CMY	46	NEGATIVE	76	TEM/CTX-M
17	IMP/CTX-M/SHV/CMY	47	NEGATIVE	77	TEM
18	IMP/TEM	48	NEGATIVE	78	CTX-M
19	IMP/TEM/CTX-M/SHV/CMY	49	NEGATIVE	79	TEM/CTX-M
20	KPC/CTX-M/SHV/CMY	50	NEGATIVE	80	CTX-M
21	IMP/TEM	51	NEGATIVE	81	CTX-M
22	IMP/TEM/CTX-M/CMY	52	TEM/CTX-M/SHV/CMY	82	CTX-M
23	IMP/TEM/SHV/CMY	53	TEM	83	CTX-M
24	IMP/TEM/CTX-M/SHV/CMY	54	TEM	84	CTX-M
25	IMP/TEM	55	TEM	85	TEM/CTX-M
26	IMP/TEM/CTX-M/SHV/CMY	56	TEM		
27	IMP/TEM/CTX-M/SHV/CMY	57	TEM		
28	IMP/TEM	58	NEGATIVE		
29	TEM	59	NEGATIVE		
30	IMP/TEM/CTX-M	60	DHA		

In order to mimic clinical samples, a swab was used to sample each of the Zeptometrix validation panel members and re-analysed. The results obtained were identical to those shown in Table 2, indicating the feasability of the assay to work with primary patient swabs.

Clinical validation

The results from testing 85 clinical ESBL/CPO isolates and controls is shown in Table 3. In summary, there was a 100% correlation with the phenotypic data generated by the hospital and the results obtained using the Genetic Signatures ESBL/CPO assay.

Conclusions

The Genetic Signatures ESBL/CPO assay provides a sensitive and specific alternative for the detection of Extended Spectrum Beta-Lactamase and Carbapenemase producing organisms. The use of multi-coloured fluorogenic probes enables the expansion of the multiplexing capabilities of standard PCR instrumentation. The assay can be carried out in less than 3 hours with minimal hands on time for laboratory technicians, allowing physicians timely treatment options for infected patients.

Genetic Signatures