

A Novel Fluorogenic Assay for Rapid Detection of Carbapenemases in Multidrug-Resistant *Enterobacteriaceae*

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BACKGROUND

In recent years, under the selective challenge of antibiotics, the variety and number of drug-resistant pathogenic microorganisms have increased significantly, which brings great challenges to clinical diagnosis and treatment, especially the infection caused by carbapenem resistant *Enterobacteriaceae* (CRE). Carbapenemases production is the main mechanism of drug resistance of *Enterobacteriaceae* to carbapenems. Drug resistance of Carbapenems can be caused by three mechanisms, resulting in the production of five major Carbapenemases. These are Klebsiella pneumoniae enzyme (KPC), New Delhi metal β -lactamase (NDM), carbapenem hydrolyzed oxalase (OXA-48 like), integrin-encoded metal β -lactamase (VIM) and IMP (para imipenem). The real-time fluorescence quantitative PCR (qPCR) method based on molecular beacons was combined with the melting curve analysis to identify five drug resistance genes simultaneously by a single PCR reaction, with rapid detection, high sensitivity and strong specificity. Based on this principle, we developed the novel fluorogenic assay for rapid detection of Carbapenemases in multidrug-resistant *Enterobacteriaceae* (Dynamiker Biotechnology (Tianjin) Co., Ltd.).

METHOD

We evaluated the performance of the novel fluorogenic assay for rapid detection of Carbapenemases in multidrug-resistant *Enterobacteriaceae*, including the limit of detection (LoD) and cross-reactivity, and compared it with the lateral flow immunochromatography assay (LFA).

RESULT

The LoD ranged from 75-450 CFU/mL for the five carbapenemase genes. The analytical specificity for target genes was 100%, as assessed with a panel of 15 pathogens, which indicated no cross-reactions. Comparison of qPCR and LFA results from twenty-three CRE clinical isolates with characterized carbapenemase content demonstrated a complete agreement (Table 1-2).

CONCLUSION

The novel fluorogenic assay for rapid detection of Carbapenemases in multidrug-resistant *Enterobacteriaceae* is an accurate and rapid method to identify KPC, NDM, VIM, IMP and OXA-48-like carbapenemases in the clinical microbiology laboratory, which can guide infection control programs to limit the spread of these organisms.

KEY WORDS

Fluorogenic Assay; Rapid detection; Carbapenem resistant Enterobacteriaceae

Table 1 Detection results of clinical isolates by the two methods between qPCR and LFA

Strain No.	Organism name	PCR	LFA	Consistency
CRE 1-1	<i>Klebsiella pneumoniae</i>	VIM-1	VIM	Y
CRE 1-2	<i>Pseudomonas aeruginosa</i>	VIM-10	VIM	Y
CRE 2-1	<i>Escherichia coli</i>	NDM-5	NDM	Y
CRE 2-2	<i>Klebsiella pneumoniae</i>	NDM-1	NDM	Y
CRE 3-1	/	KPC-2/NDM-1	KPC/NDM	Y
CRE 3-2	/	KPC-2/NDM-1	KPC/NDM	Y
CRE 4-1	<i>Enterobacter cloacae</i>	IMP-4	IMP	Y
CRE 4-2	<i>Klebsiella pneumoniae</i>	IMP-4	IMP	Y
CRE 5-1	<i>Klebsiella pneumoniae</i>	OXA-48	OXA-48	Y
CRE 5-2	<i>Klebsiella pneumoniae</i>	OXA-232	OXA-48	Y
CRE 6-1	<i>Klebsiella pneumoniae</i>	KPC-2	KPC	Y
CRE 6-2	<i>Serratia marcescens</i>	KPC-2	KPC	Y
CRE-0	<i>Serratia marcescens</i>	/	/	Y
CRE-100	<i>Klebsiella pneumoniae</i>	/	/	Y
CRE-102	<i>Klebsiella pneumoniae</i>	KPC	KPC	Y
CRE-121	<i>Klebsiella pneumoniae</i>	NDM	NDM	Y
CRE-134	<i>Escherichia coli</i>	/	/	Y
CRE-144	<i>Klebsiella pneumoniae</i>	/	/	Y
CRE-152	<i>Escherichia coli</i>	NDM	NDM	Y
CRE-176	<i>Klebsiella pneumoniae</i>	NDM	NDM	Y
CRE-227	/	NDM	NDM	Y
CRE-231	/	VIM	VIM	Y
KM174	/	NDM	NDM	Y

“Y”: Yes

Table 2 The cross-reaction results of 15 pathogens

Organism name	Strain No.	Concentration	Result
<i>Escherichia coli</i>	ATCC8739	2.67E+06	N
<i>Acinetobacter baumannii</i>	ATCC17912	2.27E+06	N
<i>klebsiella pneumoniae</i>	ATCC700603	5.20E+06	N
<i>Enterobacter cloacae</i>	ATCC13047	8.95E+06	N
<i>Candida albicans</i>	ATCC10231	5.00E+05	N
<i>Klebsiella oxytoca</i>	ATCC15328	4.76E+06	N
<i>Enterococcus faecalis</i>	ATCC29212	3.15E+06	N
<i>Staphylococcus aureus</i>	ATCC25923	4.53E+06	N
<i>Clostridium difficile</i>	ATCC43255	1.80E+07	N
<i>Bifidobacterium adolescentis</i>	ATCC15703	5.00E+05	N
<i>Enterobacter aerogenes</i>	ATCC13048	3.19E+06	N
<i>Helicobacter pylori</i>	ATCC43504	1.61E+06	N
<i>Campylobacter jejuni</i>	ATCC33560	3.27E+06	N
<i>Cryptococcus neoformans</i>	ATCC 32045	2.67E+06	N
<i>Cryptococcus gertmanni</i>	ATCC MYA-4560	2.27E+06	N

“N”: Negative