Performance of a Novel Fluorogenic Assay for Detection of Carbapenemase-producing *Enterbacteriaceae*

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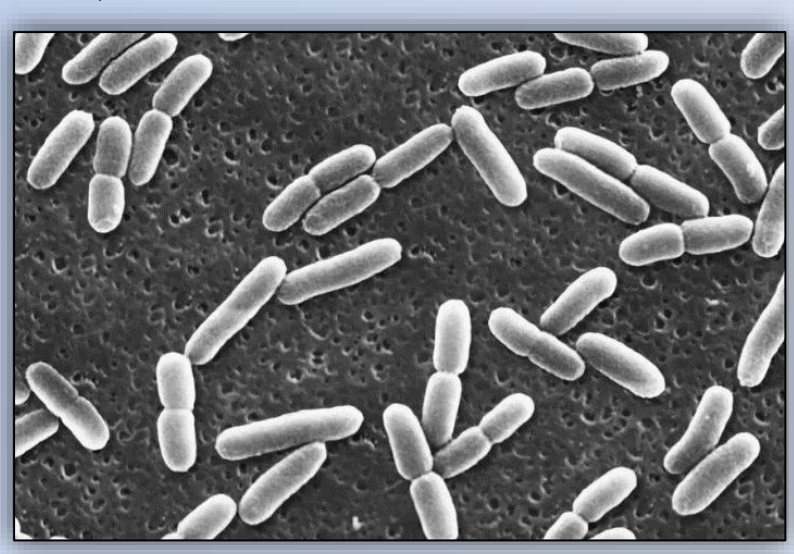
Doudou Jiang [1, 2], Shuli Li [1, 2], Zhixian Wang [1, 2], Ying Wang [1, 2], Yuan Zhang [1, 2], He Wang [1, 2], Yan Su [1, 2], Zeqi Zhou [1, 2]

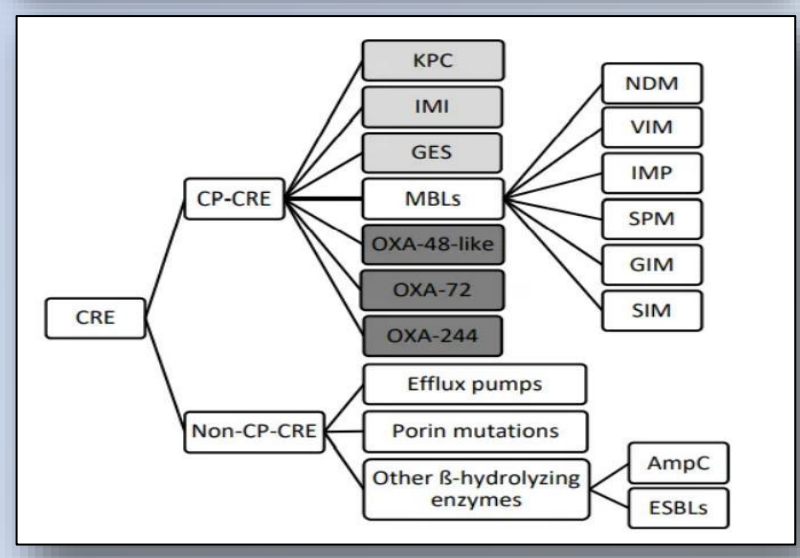
[1]Dynamiker Sub-Center of Beijing Key Laboratory for Mechanisms Research and Precision Diagnosis of Invasive Fungal Disease - Tianjin (China)

[2]Tianjin Enterprise Key Laboratory for precision diagnosis technology of invasive fungal diseases - Tianjin (China)

Backgroud

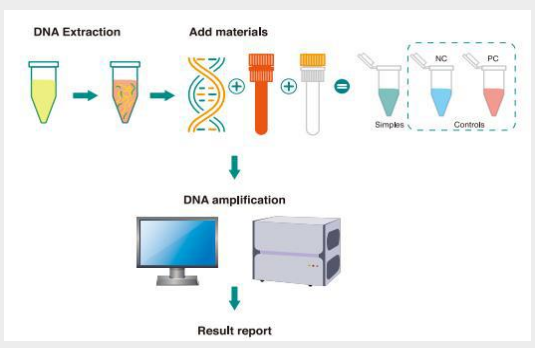
- Under the selective challenge of antibiotics, the variety and number of drugresistant pathogenic microorganisms have increased significantly, which brings great challenges to clinical diagnosis and treatment, especially the infection caused by carbapenem resistant *Enterobacteriaceae* (CRE).
- The production of carbapenemases, which are members of the β-lactamases that are divided into classes A, B and D based on their molecular structures, is the major mechanism underlying carbapenem resistance around the world and represents a great health concern. 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.
- We developed the novel fluorogenic assay for rapid detection of Carbapenemases in multidrug-resistant *Enterobacteriaceae* (Dynamiker Biotechnology (Tianjin) Co., Ltd.).

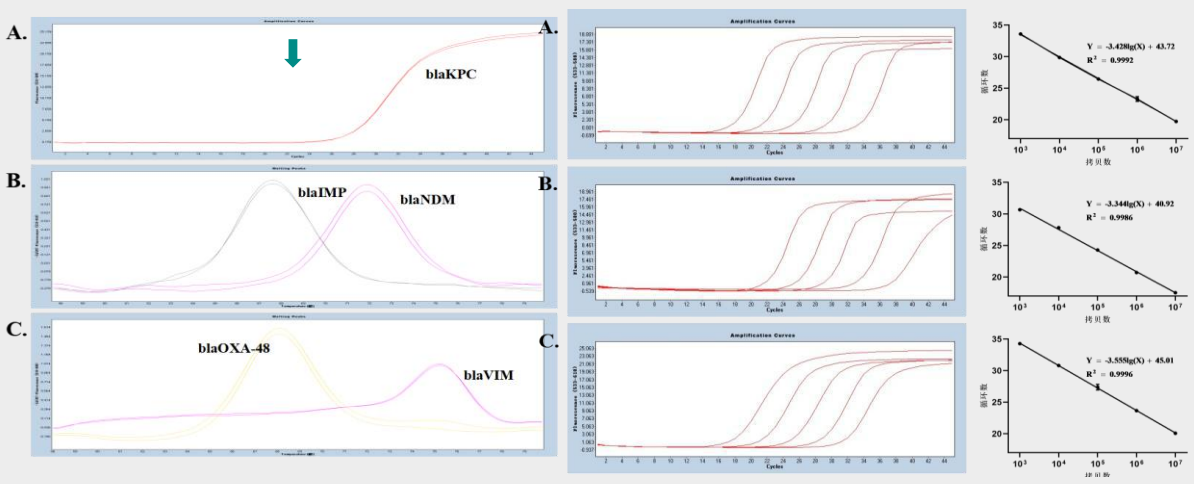




Methods

- 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).





Results

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

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	*Klebsiella pneumoniae*	VIM-1	VIM	Y
CRE 1-2	*Pseudomonas aeruginosa*	VIM-10	VIM	Y
CRE 2-1	*Escherichia coli*	NDM-5	NDM	Y
CRE 2-2	*Klebsiella pneumoniae*	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	*Enterobacter cloacae*	IMP-4	IMP	Y
CRE 4-2	*Klebsiella pneumoniae*	IMP-4	IMP	Y
CRE 5-1	*Klebsiella pneumoniae*	OXA-48	OXA-48	Y
CRE 5-2	*Klebsiella pneumoniae*	OXA-232	OXA-48	Y
CRE 6-1	*Klebsiella pneumoniae*	KPC-2	KPC	Y
CRE 6-2	*Serratia marcescens*	KPC-2	KPC	Y
CRE-0	*Serratia marcescens*	/	/	Y
CRE-100	*Klebsiella pneumoniae*	/	/	Y
CRE-102	*Klebsiella pneumoniae*	KPC	KPC	Y
CRE-121	*Klebsiella pneumoniae*	NDM	NDM	Y
CRE-134	*Escherichia coli*	/	/	Y
CRE-144	*Klebsiella pneumoniae*	/	/	Y
CRE-152	*Escherichia coli*	NDM	NDM	Y
CRE-176	*Klebsiella pneumoniae*	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.	Result
Escherichia coli	ATCC8739	N
Acinetobacter baumannii	ATCC17912	N
klebsiella pneumoniae	ATCC700603	N
Enterobacter cloacae	ATCC13047	N
Candida albicans	ATCC10231	N
Klebsiella oxytoca	ATCC15328	N
Enterococcus faecalis	ATCC29212	N
Staphylococcus aureus	ATCC25923	N
Clostridium difficile	ATCC43255	N
Bifidobacterium adolescentis	ATCC15703	N
Enterobacter aerogenes	ATCC13048	N
Helicobacter pylori	ATCC43504	N
Campylobacter jejuni	ATCC33560	N
Cryptococcus neoformans	ATCC 32045	N
Cryptococcus gertmann	ATCC MYA-4560	N

"N": Negative

Conclusions

- 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.

References

Suay-García B, Pérez-Gracia MT. Antibiotics (Basel). 2019 Aug 19;8(3):122.