00572

A Novel Fluorogenic Assay for rapid detection of Carbapenemases in multidrug-resistant *Enterobacteriaceae*

03. Bacterial susceptibility & resistance

3e. Resistance detection / prediction approaches (rapid and/or molecular assays, resistome analysis, inference methods)

Shu Li Li [1, 2], Yuan Zhang [1, 2], Zhi Xian Wang [1, 2], Xiang Yan Yan [1, 2], He Wang [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)

Background At present, bacterial drug resistance has become a major challenge in the field of global public health, 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). Currently, the standard method for detecting patients who are colonized with carbapenem-non-susceptible organisms is to culture rectal or perirectal swab samples on gram-negative selective agar plates, such as MacConkey agar, followed by antimicrobial susceptibility testing of lactose fermenting colonies, or by using selective screening agar media. The former is laborious and can require several days to generate a final result, while the latter approach varies considerably in sensitivity and specificity based on the selective medium used. The real-time fluorescence quantitative PCR 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.). (Figure1 A-B).

Methods In total, 8 clinical isolates containing KPC gene, 5 clinical isolates containing NDM gene, 5 clinical isolates containing VIM gene, 5 clinical isolates containing IMP gene and 5 clinical isolates containing OXA gene were detected by the novel fluorogenic assay. At the same time, the specific primers of each gene were designed. After PCR amplification, sequencing confirmed the data. (Figure1 C).

Results The results obtained by the real-time fluorescence quantitative PCR were consistent with the sequencing results.

Conclusions The Novel Fluorogenic Assay effective for rapid detection and identification of carbapenemases in multidrug-resistant Enterobacteriaceae, which can guide infection control programs to limit the spread of these organisms.

Figure1 Novel Fluorogenic Assay

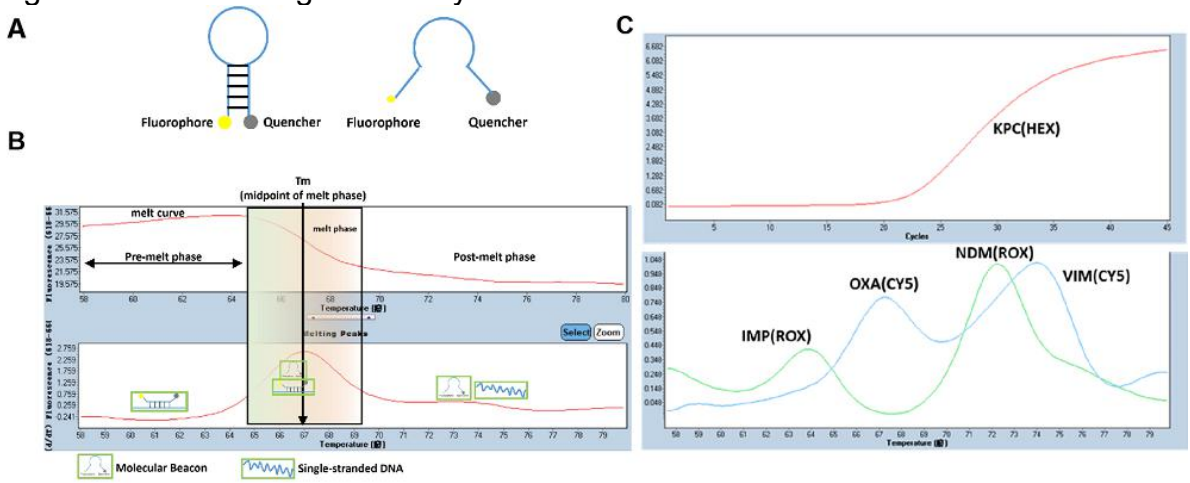

Figure1 The Novel Fluorogenic Assay. A. Molecular beacons in different states; B. Schematic diagram of fluorescence melting curve analysis with molecular beacons; C. Results of detecting 5 target genes with Dynamiker Carba-R Assay.

Keyword 1
Carbapenem resistant Enterobacteriaceae
Keyword 2
Carbapenemases
Keyword 3
PCR

Conflicts of interest

Do you have any conflicts of interest to declare?
I have no potential conflict of interest to report