"Molecular characterization of carbapenem-resistant *Klebsiella pneumoniae*"

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OXA-48-like carbapenemase gene remains a hidden threat, as different OXA-48 variants have varying presentations of susceptibility to antibiotics that might affect the treatment decisions. Rapid detection and differentiation of OXA-48-like carbapenemase genes are critical for targeted treatment and infection control. In this study, we aimed to develop high-resolution melting (HRM) analysis for the differentiation of OXA-48 variants. HRM analysis is a post-polymerase chain reaction (post-PCR) method for identification of small variations in nucleic acid sequences based on the PCR dissociation curve. A total of 82 bacterial strains, which consisted of *Enterobacteriaceae* and non-*Enterobacteriaceae,* were collected from a tertiary teaching hospital. The sensitivity and specificity of the assay were determined, and the developed assay was evaluated using the collected isolates against conventional-sequencing method. Overall, the developed assay was able to detect isolates that harboured OXA-48 and OXA232/OXA-181 by showing two distinct peaks at 81.1 ± 0.2 °C and 82.1 ± 0.2 °C, respectively. The detection limit of the assay was 1.6 x 10-6 ng/µl for OXA-48 and 1.8 x 10-7 ng/µl for OXA-232/OXA-181. This assay showed 100% specificity when evaluated on a panel of 37 isolates comprised of different species of bacteria and yeasts. When the assay with isolates collected in the year 2016 was first evaluated, the assay showed comparable results with conventional PCR-sequencing method where 34 OXA-48 and OXA-232/OXA-181 were detected. By using HRM analysis, the presence of OXA-48-like variants could be easily identified within 3 hours from the pure culture.

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