Direct detection of Carbapenemase-producing *Klebsiella pneumoniae* from Blood Culture Bottle Using a Commercial Real-time PCR Package

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**Introduction:** Early detection of carbapenem-producing *Klebsiella pneumoniae* (CRKP) is important for prompt antimicrobial therapy and effective infection control. Current conventional identification method takes ≥48 hours from positive alarm of blood culture. The use of molecular assays on the other hand is hampered by presence of the sodium polyanetholesulfonate (SPS) and other PCR inhibitors. Thus, the aim of this study was to evaluate the use of special DNA extraction method before real-time PCR amplification for early detection of carbapenamase producers from blood culture system.

**Methodology:** Twenty-eight CRKP-confirmed isolates by phenotypic test and 8 CRKP-negative isolates were spiked with 10 mls sterile whole blood into aerobic Bactec Plus bottles until the growth detected. 1ml of the blood specimen was subjected to DNA extraction following Qiagen QIAamp® Blood Mini kit protocol, Villumsen et. al protocol and modified Villumsen et. al (2010) protocol. The specimen was then amplified by multiplex real-time PCR for five known β-lactamases i.e NDM-1, OXA-48, VIM, IMP and KPC.

**Results:** The modified Villumsen et. al protocol was able to remove PCR inhibitors in blood culture system. The assay was able to amplify all 28 CRKP strains from spiked blood culture specimens and no cross amplification was observed in CRKP-negative samples. Total number of 26 CRKP strains was successfully detected and the remaining 2 strains result was false negative. Sensitivity of direct detection of CRKP was 92.9% and 100% specificity using this method.

**Conclusion:** The modified DNA extraction method was effective in removing PCR inhibitors from blood culture system that enable direct detection of CRKP from blood culture system. Further evaluation using clinical specimen is required for early diagnosis of CRKP bacteremia from suspected patients.

**Keywords:** carbapenamase, multiplex real-time PCR, direct detection