



Rapid and sensitive detection of OXA-23-mediated carbapenem resistance in *Acinetobacter baumannii* using a paper-based immunoassay with silver-based signal amplification

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Abstract

Increased incidence of infections caused by carbapenem-resistant *Acinetobacter baumannii* (CRAB) in intensive care units has necessitated the development of rapid diagnostics. Resistance is primarily caused by OXA-type carbapenemases, of which OXA-23 is the most widespread. This study describes the development of recombinant monoclonal antibodies targeting OXA-23 and their application in a paper-based assay for rapid CRAB detection. Six scFv binders were identified from an immunized phage-displayed mouse antibody library against the OXA-23 protein, of which scFv 11 and 13 exhibited high sensitivity. These clones retained their binding activity in the presence of detergent-based cell lysis buffer and demonstrated specific detection of OXA-23 with no cross-reactivity to related carbapenemases. A paper-based visual detection assay was designed using mono-biotinylated OXA scFv 11 and streptavidin-conjugated gold nanoparticles as detector molecules, with a provision for silver-based color enhancement for signal amplification. The assay enabled the detection of OXA-23-producing *A. baumannii* at a limit of 1×10^4 CFU/ml within 30 min, from culture lysis to result interpretation. This detection limit aligns with the bacterial loads typically observed in ventilator-associated pneumonia, highlighting the suitability of the assay for clinically relevant detection. The assay showed 100% sensitivity and 100% specificity when evaluated against well-characterized clinical isolates. It offers operational ease, and clinically relevant sensitivity and specificity comparable to existing immunochromatographic platforms. The silver-based color enhancement step further improves visual identification, thereby allowing the unambiguous detection of low bacterial loads without the need for complex instrumentation. The assay is expected to facilitate early resistance detection in CRAB and improve clinical decision-making.

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