O047

2-hour Oral Session

Detection of carbapenemases

Development of a novel immunochromatographic confirmatory test for the detection of OXA-48 carbapenemase in Enterobacteriaceae

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Objectives

Infections by carbapenemase-producing Enterobacteriaceae (CPE) cause a major public health concern worldwide and OXA-48 represents the most challenging resistance mechanism for diagnostic laboratories. While phenotypic confirmatory tests exist for the confirmation of Class A and Class B carbapenemases, confirmation of OXA-48 requires time consuming and/or expensive molecular analysis. We developed the OXA-48 *K*-SeT test, a new lateral flow assay that specifically detects OXA-48-like carbapenemases from bacterial colonies in less than 10 minutes.

Methods

Monoclonal antibodies were raised against OXA-48 using a DNA immunization strategy. Western blots were performed on recombinant OXA-48. Epitope mapping was initially performed by competition experiments and was then precised by testing the antibodies on overlapping peptides. Immunochromatographic sandwich tests were developed by using antibodies in both capture and detection after coupling to colloidal gold particles. The best antibody pair was selected to build a prototype test that was evaluated on recombinant OXA-48 protein and on lysates of OXA-48-producing strains.

Results

More than 20 antibodies specifically targeting recombinant OXA-48 carbapenemase were obtained after DNA immunization. These antibodies did all recognize linear epitopes as shown by Western blot analysis, and clustered into 6 groups based on their cross-competition capabilities. Epitope mapping allowed defining the smallest recognized linear peptide for each of the best reacting antibodies used either as capture or detection reagents in a sandwich assay. The best capture/detection antibodies couple was used for further characterisation. This assay (OXA-48 *K*-SeT test) is able to detect as low as 0.125 ng/ml of recombinant OXA-48 protein. The test is highly specific for the detection of OXA-48 carbapenemase producing strains, including its variants, and it does not detect any other carbapenemase types (i.e. KPC, NDM, VIM and IMP). Tests were performed directly on bacterial colonies grown on solid medium after suspension in a specific buffer. The OXA-48 *K*-SeT test was able to detect OXA-48 directly from one single colony in less than 10 minutes. The limit of detection of the test is 1.5 10^6 CFU/ml when performed on serial dilutions of a suspension of OXA-48 producing *Klebsiella pneumoniae* isolate.

Conclusion

We developed a new lateral flow assay (OXA-48 *K*-SeT test) for the rapid detection of OXA-48 carbapenemase. The assay is aimed to be used as confirmatory test in the routine diagnostic microbiology laboratory. The performance of the test requires further assessment on a larger panel of clinical bacterial isolates. The OXA-48 *K*-SeT test is the first rapid identification confirmatory non-molecular assay for OXA-48 detection with results available within minutes.